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(71) Applicant (for all designated States except US): **GILEAD SCIENCES, INC.** [US/US]; 333 Lakeside Drive, Foster City, CA 94404 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BIRKUS, Gabriel** [SK/US]; 1266 Stanyan Street #4, San Francisco, CA 94117 (US). **CHEN, James, M.** [US/US]; 4015 Marblehead Drive, San Ramon, CA 94583 (US). **CHEN, Xiaowu** [US/US]; 377 Laurie Meadows Drive, Apt. 319, San Mateo, CA 94403 (US). **CIHLAR, Tomas** [CZ/US]; 727 Caravel Lane, Foster City, CA 94404 (US). **EISENBERG, Eugene, J.** [US/US]; 236 Club Drive, San Carlos, CA 94070 (US). **HATADA, Marcos** [US/US]; 2000 Walnut Avenue, Apt. G302, Fremont, CA 94538 (US). **HE, Gong-Xin** [CN/US]; 5436 Ontario Common,

Fremont, CA 94555 (US). **KIM, Choung, U.** [US/US]; 1750 Elizabeth Street, San Carlos, CA 94070 (US). **LEE, William, A.** [US/US]; 749 Anderson Drive, Los Altos, CA 94024 (US). **MCDERMOTT, Martin, J.** [US/US]; 710 Bair Island Road, Suite 202, Redwood City, CA 94063 (US). **SWAMINATHAN, Sundaramoorthi** [IN/US]; 2858 Hillside Drive, Burlingame, CA 94010 (US).

(74) Agents: **RIEDL, Peter et al.**; Reitstöter, Kinzebach & Partner (GbR), Sternwartstrasse 4, 81679 München (DE).

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(54) Title: METHOD AND COMPOSITIONS FOR IDENTIFYING ANTI-HIV THERAPEUTIC COMPOUNDS

(57) Abstract: The invention relates to methods and compositions for identifying compounds having therapeutic activity against human immunodeficiency virus (HIV).

METHOD AND COMPOSITIONS FOR IDENTIFYING
ANTI-HIV THERAPEUTIC COMPOUNDS

This non-provisional application claims the benefit of Provisional Application No. 60/375,622, filed April 26, 2002, Provisional Application No. 60/375,779 filed April 26, 2002, Provisional Application No. 60/375,834 filed April 26, 2002 and Provisional Application No. 60/375,665 filed April 26, 2002, which are incorporated herein by reference. Additionally, PCT applications PCT/US 03/12901 and PCT/US 03/12926 are incorporated herein by reference in their entirety.

Field of the Invention

10 The invention relates generally to methods and compositions for identifying compounds having therapeutic activity against human immunodeficiency virus (HIV).

Background of the Invention

Anti-HIV compounds are well established and have achieved significant therapeutic benefit. However, existing therapeutics remain less than optimal. Conspiring to reduce patient compliance and therapeutic efficacy are toxicity, resistant HIV, poor bioavailability, low potency, and frequent and inconvenient dosing schedules, among other failings. The need to administer very large tablets and requirements for frequent dosing characterize a number of important anti-HIV therapeutics, most particularly the HIV protease inhibitors. While significant advances have been made in preparing improved nucleotide analogue anti-HIV therapeutics (see WO 02/08241, EP 820,461 and WO 95/07920, all of which are hereby incorporated by reference), other anti-HIV therapeutic drug classes remain encumbered with severe deficiencies.

Summary of the Invention

25 The present invention provides methods and compositions for identifying therapeutic anti-HIV compounds having improved pharmacological and therapeutic properties. In particular, this invention provides for novel candidate therapeutic anti-HIV compounds and methods for screening them to identify compounds having such beneficial properties.

In accordance with this invention, a method is provided that comprises

- 30 (a) identifying a non-nucleotide prototype compound;
- (b) substituting the prototype compound with an esterified carboxyl or esterified phosphonate-containing group to produce a candidate compound; and
- (c) determining the anti-HIV activity of the candidate compound.

In another embodiment, a method is provided that comprises

- (a) selecting a non-nucleotide candidate compound containing at least one esterified carboxyl or esterified phosphonate-containing group and
- (b) determining the intracellular persistence of the candidate compound or a esterolytic metabolite of the esterified carboxyl or phosphonate-containing group thereof.

In a further embodiment, determining the anti-HIV activity of the candidate compound comprises determining the anti-HIV activity of a carboxylic acid or phosphonic acid-containing metabolite of the candidate compound, which carboxyl acid or phosphonic acid-containing metabolite is produced by esterolytic metabolic cleavage of the esterified carboxyl or phosphonate-containing group. In another embodiment determining anti-HIV activity comprises determining the the tissue selectivity and/or the intracellular residence time of at least one of said intracellular carboxylic acid or phosphonic acid-containing metabolites.

In another embodiment of this invention, a library of anti-HIV candidate compounds is provided that comprises at least one non-nucleotide prototype compound substituted by an esterified carboxyl or phosphonate group. Such libraries facilitate large-scale screening of candidate compounds.

This invention is an improvement in the conventional methods for identifying therapeutic anti-HIV compounds. Thus, in a method for identifying an anti-HIV therapeutic compound, the improvement comprises substituting a prototype compound with an esterified carboxyl or phosphonate and assaying the resulting candidate compound for its anti-HIV activity.

Adding the esterified carboxyl or phosphonate group to the prototype molecule produces significant advantages in the pharmacologic properties of the prototype. Without being held to any particular method of operation of the invention, it is believed that the ester(s) mask the charge of the carboxyl or phosphonate and permit the candidate to enter HIV infected cells, in particular peripheral blood mononuclear cells (PBMCs). Once the candidate has entered the cells it is processed by biological mechanisms (most notably, it is believed, by a newly discovered PBMC enzyme which we designate GS-7340 Ester Hydrolase) to produce at least one metabolite containing a free carboxylic acid and/or phosphonic acid. This metabolite is antivirally active against HIV. These charged metabolic depot forms are exceptionally

persistent in the cells, thereby permitting substantial reductions in the frequency of dosing compared to the parental prototype, among other advantages. In addition, the esterified carboxyl or phosphonate substituent may direct the selective distribution of the prototype to tissues (most particularly lymphoid tissues such as PBMCs) which are noted sites of HIV infection, thereby potentially reducing systemic dose and toxicity.

In further embodiments, assaying for anti-HIV activity optionally comprises screening the candidate compounds for their susceptibility to esterolytic cleavage by isolated GS-7340 Ester Hydrolase. The isolated Hydrolase is a further embodiment of this invention.

Since GS-7340 Ester Hydrolase may interact with other compounds than the anti-HIV candidates, it will be of pharmacologic utility to determine if the enzyme is cleaving such other compounds. Thus, another embodiment of this invention is a method comprising obtaining a substantially pure organic molecule, optionally contacting the organic molecule with another molecule to produce a composition, contacting GS-7340 Ester Hydrolase with said organic molecule or composition, and optionally determining whether the organic molecule has been cleaved by the Hydrolase.

In another embodiment, a method is provided comprising contacting GS-7340 Ester Hydrolase with an organic compound in a cell-free environment.

In a further embodiment, a method is provided comprising contacting GS-7340 Ester Hydrolase with an organic compound in an *in vitro* or cell culture environment.

In another embodiment, a composition is provided comprising a substantially pure organic compound and isolated GS-7340 Ester Hydrolase.

In another embodiment, a composition is provided comprising an organic compound and GS-7340 Ester Hydrolase in an *in vitro* or cell culture environment.

These and other embodiments of this invention are more fully described in the following disclosure.

Detailed Description of the Invention

The following disclosure contains detailed embodiments of the practice of the invention. These are provided to more fully describe the invention, but the invention is not limited to these embodiments.

“Anti-HIV activity” of candidates is determined by any method for assaying the HIV inhibitory activity of a substance. Many such methods are well known, and range from *in vitro* enzyme assays (e.g., HIV reverse transcriptase or integrase assays) to animal studies (e.g., SIV in chimps) and human clinical trials. Included with this term are any assays bearing on the therapeutic anti-HIV efficacy of a substance, e.g., HIV resistance determinations, biodistribution, and intracellular persistence.

“Candidate compound” is an organic compound containing an esterified carboxylate or phosphonate. Optionally, candidate compounds excluded compounds heretofore known to have anti-HIV activity. With respect to the United States, the candidate compounds herein exclude compounds that are anticipated under 35 USC §102 or obvious under 35 USC §103 over the prior art. In other jurisdictions using the novelty and inventive step criteria, the candidate compounds exclude compounds not novel or which lack inventive step over the prior art. However, libraries containing candidate compounds optionally comprise known compounds. These may be, for example, reference compounds having known anti-HIV activity.

“Non-nucleotide” means any compound that has all of the following characteristics: It does not already contain an esterified carboxyl or phosphonate, it is not a phosphonate or phosphate-containing compound disclosed in WO 02/08241, EP 820,461 or WO 95/07920 and it does not already contain a phosphonate group. GS-7340 is an example of a nucleotide anti-HIV compound. Many other examples of such compounds are known. These compounds are excluded from the scope of prototype compounds and are not employed in the candidate compound screening method or candidate compound compositions of this invention. For the most part, the nucleotide analogues comprise the substructure $-\text{OC}(\text{H})_2\text{P}(\text{O})=$ coupled (usually at the 9 position of purine bases or the 1 position of pyrimidine bases) via a sugar or cyclic or acyclic sugar analogue (aglycon) to a nucleotide base or an analogue thereof. The base analogues typically are substituted, usually at extracyclic N atoms, or are the aza or deaza analogues of the naturally occurring base scaffolds. They are fully set forth in the above

described art and are well known in the field. See for example U.S. Patent 5,641,763 and related patents and publications by Antonin Holy.

Optionally excluded from the scope of the libraries of this invention are any phosphonates disclosed by WO99/33815, WO99/33792, WO99/33793, WO00/76961 and their related, progeny and parental filings, all of which are hereby incorporated by reference. However, unless expressly excluded by the claims herein, such compounds shall be considered candidate compounds. Further, the act of making and screening the phosphonates of such filings to determine their intracellular persistence (whether by preclinical assays such as that using GS-7340 Ester Hydrolase, or by clinical studies) falls within the scope hereof, as does obtaining regulatory approval to market one of them and selling the selected phosphonate.

"Non-nucleoside" means any compound that is not a nucleotide base linked to a sugar or aglycon (cyclic or acyclic) and terminating at the 5' position (or the analogous position in nucleosides containing sugar analogues) by hydroxyl or a group which is metabolized *in vivo* to hydroxyl. The nucleosides are distinguishable from the nucleotides in not containing a phosphate or, in the case of relevant nucleotide analogues, a phosphonate.

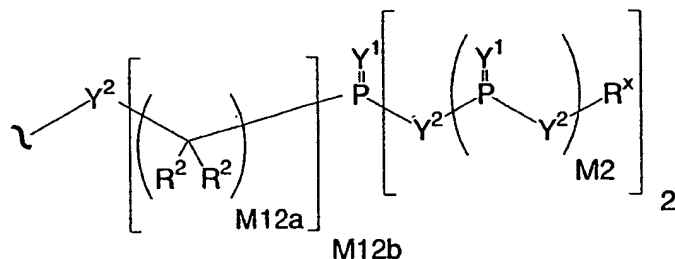
"Phosphonate-containing group" is a group comprising a phosphorus atom singly bonded to carbon, double bonded to oxygen and singly bonded to two other groups through oxygen, sulfur, or nitrogen. In general, the carbon bond is to a carbon atom of the prototype or a linking group to the prototype and the single bonds to oxygen, nitrogen or sulfur are bonds to oxy or thioesters or are amino acid amidates in which the terminal carboxyl group(s) are esterified.

"Carboxyl-containing groups" are any group having a free carboxyl serving as the site for esterification. An "organic acid" is any compound containing carboxyl and at least one additional carbon atom.

The "esterified carboxyl or esterified phosphonate group" is any group capable of intracellular processing to yield a free carboxyl and/or free phosphonic acid. The structure of these groups is not important other than that the free acid be produced intracellularly. Preferably, systemic or digestive esterolysis is minimized in preference to intracellular

hydrolysis. This permits maximum migration of the candidate into target cells and maximum intracellular retention of the acid metabolites.

Suitable exemplary esterified carboxyl or phosphonate groups are described herein. Others are identified by screening for esterolysis *in vivo*, in PBMCs or using GS-7340 Ester Hydrolase. These groups have the structure A³, wherein A³ is a group of the formula

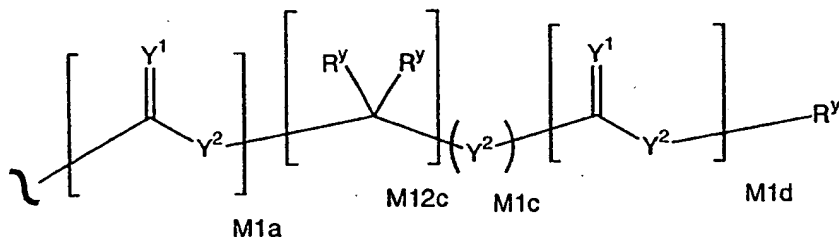


in which:

Y¹ is independently O, S, N(R^x), N(O)(R^x), N(OR^x), N(O)(OR^x), or N(N(R^x)(R^x));

Y² is independently a bond, O, N(R^x), N(O)(R^x), N(OR^x), N(O)(OR^x), N(N(R^x)(R^x)), -S(O)_{M2}⁻, or -S(O)_{M2}-S(O)_{M2}⁻;

R^x is independently H, W³, a protecting group, or a group of the formula:



R^y is independently H, W³, R² or a protecting group;

R¹ is independently H or alkyl of 1 to 18 carbon atoms;

R² is independently H, R³ or R⁴ wherein each R⁴ is independently substituted with 0 to 3 R³ groups;

R³ is R^{3a}, R^{3b}, R^{3c} or R^{3d}, provided that when R³ is bound to a heteroatom, then R³ is R^{3c} or R^{3d};

R^{3a} is F, Cl, Br, I, -CN, N₃ or -NO₂;

R^{3b} is Y¹;

R^{3c} is -R^x, -N(R^x)(R^x), -SR^x, -S(O)R^x, -S(O)₂R^x, -S(O)(OR^x), -S(O)₂(OR^x), -OC(Y¹)R^x, -OC(Y¹)OR^x, -OC(Y¹)(N(R^x)(R^x)), -SC(Y¹)R^x, -SC(Y¹)OR^x, -SC(Y¹)(N(R^x)(R^x)), -N(R^x)C(Y¹)R^x, -N(R^x)C(Y¹)OR^x, or -N(R^x)C(Y¹)(N(R^x)(R^x));

R^{3d} is $-C(Y^1)R^x$, $-C(Y^1)OR^x$ or $-C(Y^1)(N(R^x)(R^x))$;

R^4 is an alkyl of 1 to 18 carbon atoms, alkenyl of 2 to 18 carbon atoms, or alkynyl of 2 to 18 carbon atoms;

R^5 is R^4 wherein each R^4 is substituted with 0 to 3 R^3 groups;

R^{5a} is independently alkylene of 1 to 18 carbon atoms, alkenylene of 2 to 18 carbon atoms, or alkynylene of 2-18 carbon atoms any one of which alkylene, alkenylene or alkynylene is substituted with 0-3 R^3 groups;

W^3 is W^4 or W^5 ;

W^4 is R^5 , $-C(Y^1)R^5$, $-C(Y^1)W^5$, $-SO_2R^5$, or $-SO_2W^5$;

W^5 is carbocycle or heterocycle wherein W^5 is independently substituted with 0 to 3 R^2 groups;

$M2$ is 0, 1 or 2;

$M12a$ is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

$M12b$ is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

$M1a$, $M1c$, and $M1d$ are independently 0 or 1; and

$M12c$ is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12.

The esterified group is attached to the prototype through a bond or via intermediary linking groups such as the A^1 subgroup $-[Y^2-(C(R^2)_2)_{m12a}]_{m12b}Y^2W^6$ - defined below.

Candidates optionally are substituted with a single substituent which contains both an esterified carboxyl and an esterified phosphonate. In addition, or as an alternative, the candidate contains separate substituents bearing esterified carboxyl and/or phosphonate groups. An example of a combined group would a phosphonate in which a free valence of the phosphorus atom is bonded to the hydroxy of an hydroxyorganic acid or to the amino group of an amino acid wherein the carboxyl groups of the organic acid or amino acid are esterified.

"Esterified" means that the phosphonate or carboxyl is bonded to a carbon atom-containing group through oxygen or sulfur, as in $-P(O)(OR)-$ or $-COOR$ for example, where R is a carbon containing group such as alkyl or aryl.

"Protecting group" is a group covalently bonded to a labile site on the candidate compound, which site is expected to be labile under the conditions to be encountered by the

candidate, for example during synthetic procedures, during exposure to ambient conditions, and the conditions found in *in vivo* environments. The protecting group serves to prevent degradation or otherwise undesired conversions at the labile site. Extensive disclosure of various exemplary protecting groups is found *infra*.

"Intracellular depot metabolite" is an esterolytic metabolite of the esterified carboxyl or phosphonate whereby a charged carboxyl or phosphonic acid is revealed. An example is Metabolite X, further described in the examples.

"Tissue selectivity" of candidate compounds is determined by procedures set forth in WO02/08241. The object of this determination is to find whether or not the candidate (and by extension its depot forms) are enriched in one tissue or another. It is expected that compounds containing the carboxyl or phosphonate groups as described herein will be preferentially enriched in lymphoid tissue such as PBMCs.

"Intracellular residence time," "intracellular persistence," "intracellular half life" and the like refers to a measure of the time that a candidate molecule or its anti-HIV active metabolite is found within a given cell after introduction of the esterified candidate into the cell. Any technique is suitable that demonstrates how long a candidate or its anti-HIV active metabolite(s) remain in a cell. Further description of suitable assay procedures are set forth *infra*. Ideally, the method for measuring residence time will measure the retention time of the metabolite at a concentration adequate to inhibit HIV.

A "prototype compound" is any organic compound. In general, in the method of this invention one will select prototype compounds having known structures and synthesis routes in order to reduce the synthetic burden and development costs. Typically, the prototype compound will be one that has, or at least is suspected, to have anti-HIV activity. However, since the prototype compound is serving only as a starting point for preparing candidate compounds to be screened, it is not essential that it have, or be known or suspected to have, preexisting anti-HIV activity. The prototype compound need not be published or known generally to the public. In fact, the method of this invention is advantageously practiced in ongoing proprietary research programs where anti-HIV compounds are continually identified and optimized. It also should be understood that identification or selection of the prototype compound need not be temporally related to that of the candidate compound. This means that

the prototype might be identified after one or more related candidate compounds are made, or the prototype might be an early version of a compound class that has advanced further into development before the candidate based on the early prototype is actually synthesized. The prototype compound also may be entirely conceptual or may be in various phases of development. No actual prototype need to have been made, nor tested for activity or any other properties. This is often the case with candidates that are the product of truncating an existing compound and then inserting a linker group in place of all or a part of the omitted portion. In addition, it is not necessary that the prototype compound be conceived independently of the esterified substituent, i.e., it is not necessary to have the prototype in mind before designing the esterified substitution. The conception of the candidate compound optionally is a single act. Of course, the candidate compound may be based on a prototype which is in fact a previously made candidate compound and the subsequent candidate is multiply substituted with the carboxyl or phosphonate ester. Also, it will be understood that a candidate or group of candidates compounds optionally are based on an original prototype even though intervening candidates or libraries of candidates have been made.

The prototypes generally serve as the starting point for designing and identifying candidate compounds. Generally a prototype will not contain a phosphonate or carboxyl group, but it may do so if the phosphonate or carboxyl are not esterified (since candidates contain esterified phosphonate or carboxyl groups). It is most efficient to start with prototypes already known to have anti-HIV activity (preferably compounds active against anti-HIV protease, HIV integrase or HIV polymerase), but it is not essential to do so. For example, a prototype optionally is a subsegment or fragment of a compound known to possess anti-HIV activity, even though the fragment need not be active against HIV in its own right. In this instance, the phosphonate or carboxyl group restores anti-HIV activity to the candidate.

"Linker" or "link" is a bond or an assembly of atoms binding the prototype to the the esterified phosphonate or carboxyl-containing group. The nature of the linker is not critical. The linker need not be involved in the interactions of the esterified carboxyl or phosphonate group with GS-7340 Ester Hydrolase or other processing enzymes, nor need it be involved in the therapeutic interaction of the prototype with its target protein. This is not to say that these functions could not be enhanced or influenced by the linker, but it is not necessary that the linker perform or contribute to such functions. Thus, it is a straight-forward matter of

elemental organic chemistry to devise suitable linker groups and methods for joining the esterified groups.

Some general principles are useful in selecting suitable linker groups, despite their lack of criticality. First, they will not be so bulky as to interfere with the interaction of the remainder of the prototype with its target protein, e.g., HIV protease inhibitor, nor will they bear reactive or unstable groups once the linkage has been accomplished. Such chemically reactive groups will be well known to the artisan, and the parameters of bulky linkers can be evaluated by molecular modeling. Resources are available to model proteins involved in a number of diseases and disorders of lymphoid tissues, in particular HIV protease. In general, the linker will be relatively small, on the order of about 16-500 MW, typically about 16-250, ordinarily about 16-200, although as noted the linker can be as small as a bond. It generally will be substantially linear, containing less than about 40% of the total MW of the linker atoms being found in branching groups, typically less than 30% and ordinarily less than about 20%.

The backbone of such linker groups ideally will not contain any atom that is known to be labile to cleavage by biological processes or otherwise subject to hydrolysis in biological fluids. Typical suspect groups would be esters or amides in the backbone of the linker. The object is for the carboxyl or phosphonate to survive intracellular processing, with only the ester(s) being hydrolyzed, and the presence of labile groups in the backbone would jeopardize this function. However, if enzymatic access to labile atoms or groups is sterically hindered, e.g., by a cycloalkyl group or branched alkyl group, then labile sites optionally may be used in the linker. Labile groups also optionally can be found in locations other than backbone positions, e.g. on branching groups or cyclic substituents, where their potential cleavage would not result in the loss of the free acid functionality. Backbone alkyls, alkyl ethers (S or O), or alkyl containing N in any oxidation state are usually satisfactory. Generally the linker backbone is linear rather than branched or cyclic (although it may be desired to use branching or cyclic backbones when multiple esterified groups are substituted onto the prototype). The linker generally is chosen to permit substantial rotational freedom to the esterified group, and for this reason backbone double or triple bonds are not favored unless it is expected that they would be metabolized to less rotationally confined structures *in vivo* (e.g., oxidized to hydroxyl substituents). If it is desired to avoid interactions with the target protein then the linker optimally will have neither highly charged nor strongly hydrophobic character, although as noted such properties can have advantages in enhancing anti-HIV activity.

The typical linker to phosphonate will comprise at least the group $-\text{OCH}_2-$ (wherein the carbon is linked to the phosphorous atom), but many others will be apparent to the artisan or are described elsewhere herein.

Synthetic ease optionally will play a role in selection of the linker. For this reason, many linkers will contain a backbone or chain heteroatom such as 1 to 3 S, N or O. However, occasionally the prototype compound will contain a convenient site for insertion of the linker, e.g., a pendant hydroxyl, thus enabling a small linker group because the phosphorous atom can be linked directly, or virtually directly, to the prototype. Synthetic routes also can be devised readily that permit direct linkage of the phosphorous atom to the prototype, in which case the linker is merely a bond.

The linker optionally is grafted onto the prototype, or the prototype compound is optionally is modified to remove group(s) which then are replaced with linker(s). This may facilitate the synthesis of the candidate compound or, in some instances, may fortuitously improve the properties of the candidate. This may or may not be more efficient than simply grafting A^3 onto the prototype.

Typically, the starting point in devising a facile synthetic route for a candidate compound is to analyze the synthons employed in known methods for preparing the remainder of the prototype compound, concentrating on synthons which could contribute at least a part of the esterified group. Such synthons optionally are modified to contain the esterified group or a portion thereof (e.g., the acid, which is then esterified in a later step). They are then introduced into the remainder of the candidate in substantially the same fashion as the prototype or antecedent compound. Alternatively, a reactive group is introduced into the synthon before it is assembled into the precursor, and it is this group that is reacted with an intermediate for the carboxyl or phosphonate group. If necessary, suitable protecting groups are employed to facilitate the synthesis.

The site for insertion of the esterified carboxyl or phosphonate group on the prototype will vary widely. The esterified group preferably is substituted at any location on the prototype that does not bind substantially with the target protein or affect the functioning of a group that does interact with the target protein. These sites are identified by molecular

modeling, by consulting systematic SAR studies or by preparing pilot candidate compounds. However, it is also within the scope of this invention to insert the esterified groups at a site which is involved in binding the prototype to the target protein. Such sites optionally are used if (a) the linker reasonably replicates the function of the group on the prototype that it is displacing, e.g., it possesses a side chain containing the group, (b) if the loss in binding affinity is not critical to the functioning of the prototype or (c) if other substituents are introduced into the prototype that compensate for any loss in activity caused by the insertion of the linker.

The linker generally will contain at least two free valences (1 for the prototype and 1-3 for the esterified groups). Multivalent linkergroups can be employed to form a cyclic structure, being joined at 2 or more sites on the prototype and forming a bridge, the bridge in turn being substituted with one or more esterified carboxyl or phosphonate groups or including at least one atom encompassed within such groups. In addition, the linker does not need to be bound to the esterified group and/or the remainder of the prototype by a covalent bond, nor need it consist solely of covalently bonded atoms. Any bond meeting the basic criteria herein will be satisfactory, as for example linkage by chelation or other stable non-covalent attachment systems are included within the scope of the term "bond" as used herein.

Linkers also include polymers, e.g., those containing repeating units of alkyloxy (e.g. polyethylenoxy, PEG, polymethyleneoxy) and/or alkylamino (e.g. polyethyleneamino, Jeffamine™). Other linker groups include diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

Suitable linker groups optionally are prescreened by testing model candidates in the same fashion set forth herein for disclosed candidate compounds, e.g., screening using the Ester Hydrolase described herein, or by studying the effect of a model linker-containing candidate compound in PBMCs.

Typical linkers have the A¹ substructure $-[Y^2-(C(R^2)_2)_{m12a}]_{m12b}Y^2W^6$ -wherein Y², R², m12a and m12b are defined elsewhere herein, W⁶ is W³ having from 1 to 3 free valences and the prototype is bound to the Y² with free valence. However, many other structures would be apparent to the ordinary artisan and can be prepared by conventional means using the guidance herein.

Defined Chemical Terms

"Alkyl" is C₁-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, -CH₃), ethyl (Et, -CH₂CH₃), 1-propyl (*n*-Pr, *n*-propyl, -CH₂CH₂CH₃), 2-propyl (*i*-Pr, *i*-propyl, -CH(CH₃)₂), 1-butyl (*n*-Bu, *n*-butyl, -CH₂CH₂CH₂CH₃), 2-methyl-1-propyl (*i*-Bu, *i*-butyl, -CH₂CH(CH₃)₂), 2-butyl (*s*-Bu, *s*-butyl, -CH(CH₃)CH₂CH₃), 2-methyl-2-propyl (*t*-Bu, *t*-butyl, -C(CH₃)₃), 1-pentyl (*n*-pentyl, -CH₂CH₂CH₂CH₂CH₃), 2-pentyl (-CH(CH₃)CH₂CH₂CH₃), 3-pentyl (-CH(CH₂CH₃)₂), 2-methyl-2-butyl (-C(CH₃)₂CH₂CH₃), 3-methyl-2-butyl (-CH(CH₃)CH(CH₃)₂), 3-methyl-1-butyl (-CH₂CH₂CH(CH₃)₂), 2-methyl-1-butyl (-CH₂CH(CH₃)CH₂CH₃), 1-hexyl (-CH₂CH₂CH₂CH₂CH₂CH₃), 2-hexyl (-CH(CH₃)CH₂CH₂CH₂CH₃), 3-hexyl (-CH(CH₂CH₃)(CH₂CH₂CH₃)), 2-methyl-2-pentyl (-C(CH₃)₂CH₂CH₂CH₃), 3-methyl-2-pentyl (-CH(CH₃)CH(CH₃)CH₂CH₃), 4-methyl-2-pentyl (-CH(CH₃)CH₂CH(CH₃)₂), 3-methyl-3-pentyl (-C(CH₃)(CH₂CH₃)₂), 2-methyl-3-pentyl (-CH(CH₂CH₃)CH(CH₃)₂), 2,3-dimethyl-2-butyl (-C(CH₃)₂CH(CH₃)₂), 3,3-dimethyl-2-butyl (-CH(CH₃)C(CH₃)₃).

"Alkenyl" is C₂-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp*² double bond. Examples include, but are not limited to: ethylene or vinyl (-CH=CH₂), allyl (-CH₂CH=CH₂), cyclopentenyl (-C₅H₇), and 5-hexenyl (-CH₂CH₂CH₂CH₂CH=CH₂).

"Alkynyl" is C₂-C₁₈ hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, *sp* triple bond. Examples include, but are not limited to: acetylenic (-C≡CH) and propargyl (-CH₂C≡CH),

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene (-CH₂-), 1,2-ethyl (-CH₂CH₂-), 1,3-propyl (-CH₂CH₂CH₂-), 1,4-butyl (-CH₂CH₂CH₂CH₂-), and the like.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a

parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene (-CH=CH-).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene (-C≡C-), propargyl (-CH₂C≡C-), and 4-pentynyl (-CH₂CH₂CH₂C≡CH-).

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp³ carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g. the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, -X, -R, -O⁻, -OR, -SR, -S⁻, -NR₂, -NR₃, =NR, -CX₃, -CN, -OCN, -SCN, -N=C=O, -NCS, -NO, -NO₂, =N₂, -N₃, NC(=O)R, -C(=O)R, -C(=O)NRR, -S(=O)₂O⁻, -S(=O)₂OH, -S(=O)₂R, -OS(=O)₂OR, -S(=O)₂NR, -S(=O)R, -OP(=O)O₂RR, -P(=O)O₂RR, -P(=O)(O⁻)₂, -P(=O)(OH)₂, -C(=O)R, -C(=O)X, -C(S)R, -C(O)OR, -C(O)O⁻, -C(S)OR, -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR, -C(NR)NRR, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently -H, alkyl, aryl, heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups may also be similarly substituted.

"Heterocycle" as used herein includes by way of example and not limitation these heterocycles described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry"

(W.A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolyl, isoquinolyl, benzimidazolyl, piperidinyl, 4-piperidonyl, pyrrolidinyl, 2-pyrrolidonyl, pyrrolinyl, tetrahydrofuranyl, tetrahydroquinolyl, tetrahydroisoquinolyl, decahydroquinolyl, octahydroisoquinolyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indoliziny, isoindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinoliziny, phthalazinyl, naphthyridinyl, quinoxaliny, quinazoliny, cinnoliny, pteridinyl, 4aH-carbazolyl, carbazolyl, β -carboliny, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazoliny, pyrazolidinyl, pyrazoliny, piperazinyl, indoliny, isoindoliny, quinuclidinyl, morpholiny, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazoliny, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at

position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β -carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetetyl, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

"Carbocycle" means a saturated, unsaturated or aromatic ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g. arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, phenyl, spiryl and naphthyl.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

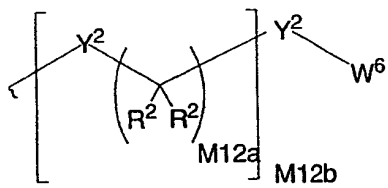
Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., McGraw-Hill Dictionary of Chemical Terms (1984) McGraw-Hill Book Company, New

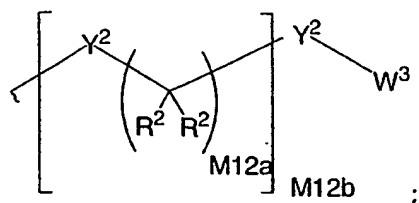
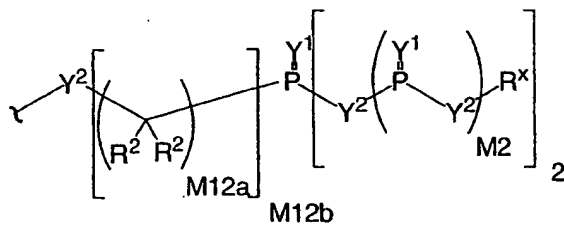
York; and Eliel, E. and Wilen, S., Stereochemistry of Organic Compounds (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and the linker or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and the linker or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Whenever a compound described herein is substituted with more than one of the same designated group, e.g., "R¹" or "R^{6a}", then it will be understood that the groups may be the same or different, i.e., each group is independently selected.

Candidate compounds contain at least one A¹ (which in turn contains 1-3 A³ groups) but also may contain at least one A² group.

A¹ is:

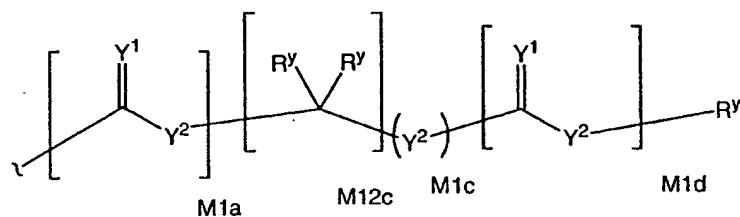


A² is:A³ is:

Y¹ is independently O, S, N(R^x), N(O)(R^x), N(OR^x), N(O)(OR^x), or N(N(R^x)(R^x));

Y² is independently a bond, O, N(R^x), N(O)(R^x), N(OR^x), N(O)(OR^x), N(N(R^x)(R^x)), -S(O)_{M2}⁻, or -S(O)_{M2}-S(O)_{M2}⁻;

R^x is independently H, R¹, W³, a protecting group, or the formula:



R^y is independently H, W³, R² or a protecting group;

R¹ is independently H or an alkyl of 1 to 18 carbon atoms;

R² is independently H, R¹, R³ or R⁴ wherein each R⁴ is independently substituted with 0 to 3 R³ groups;

R³ is R^{3a}, R^{3b}, R^{3c} or R^{3d}, provided that when R³ is bound to a heteroatom, then R³ is R^{3c} or R^{3d};

R^{3a} is F, Cl, Br, I, -CN, N₃ or -NO₂;

R^{3b} is Y¹;

R^{3c} is -R^x, -N(R^x)(R^x), -SR^x, -S(O)R^x, -S(O)₂R^x, -S(O)(OR^x), -S(O)₂(OR^x), -OC(Y¹)R^x, -OC(Y¹)OR^x, -OC(Y¹)(N(R^x)(R^x)), -SC(Y¹)R^x, -SC(Y¹)OR^x, -SC(Y¹)(N(R^x)(R^x)), -N(R^x)C(Y¹)R^x, -N(R^x)C(Y¹)OR^x, or -N(R^x)C(Y¹)(N(R^x)(R^x));

R^{3d} is -C(Y¹)R^x, -C(Y¹)OR^x or -C(Y¹)(N(R^x)(R^x));

R^4 is an alkyl of 1 to 18 carbon atoms, alkenyl of 2 to 18 carbon atoms, or alkynyl of 2 to 18 carbon atoms;

R^5 is R^4 wherein each R^4 is substituted with 0 to 3 R^3 groups;

W^3 is W^4 or W^5 ;

W^4 is R^5 , $-C(Y^1)R^5$, $-C(Y^1)W^5$, $-SO_2R^5$, or $-SO_2W^5$;

W^5 is carbocycle or heterocycle wherein W^5 is independently substituted with 0 to 3 R^2 groups;

W^6 is W^3 independently substituted with 1, 2, or 3 A^3 groups;

W^7 is a heterocycle bonded through a nitrogen atom of said heterocycle and independently substituted with 0, 1 or 2 A^0 groups;

M2 is 0, 1 or 2;

M12a is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

M12b is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12;

M1a, M1c, and M1d are independently 0 or 1; and

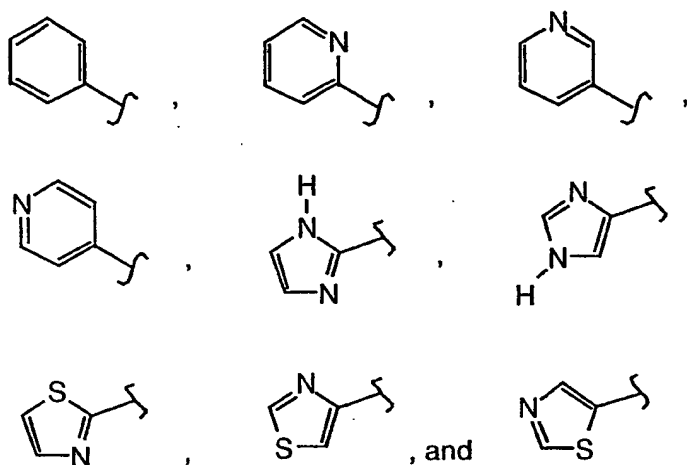
M12c is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12.

W^5 carbocycles and W^5 heterocycles may be independently substituted with 0 to 3 R^2 groups. W^5 may be a saturated, unsaturated or aromatic ring comprising a mono- or bicyclic carbocycle or heterocycle. W^5 may have 3 to 10 ring atoms, e.g., 3 to 7 ring atoms. The W^5 rings are saturated when containing 3 ring atoms, saturated or mono-unsaturated when containing 4 ring atoms, saturated, or mono- or di-unsaturated when containing 5 ring atoms, and saturated, mono- or di-unsaturated, or aromatic when containing 6 ring atoms.

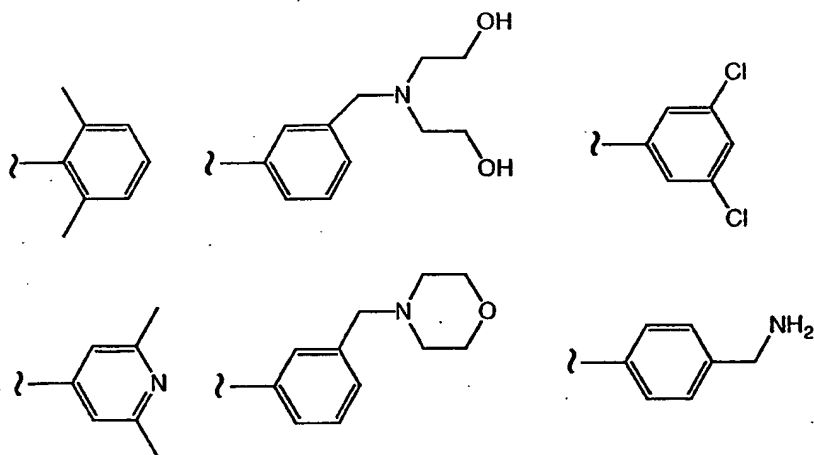
A W^5 heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S). W^5 heterocyclic monocycles may have 3 to 6 ring atoms (2 to 5 carbon atoms and 1 to 2 heteroatoms selected from N, O, and S); or 5 or 6 ring atoms (3 to 5 carbon atoms and 1 to 2 heteroatoms selected from N and S). W^5 heterocyclic bicycles have 7 to 10 ring atoms (6 to 9 carbon atoms and 1 to 2 heteroatoms selected from N, O, and S) arranged as a bicyclo [4,5], [5,5], [5,6], or [6,6] system; or 9 to 10 ring atoms (8 to 9 carbon atoms and 1 to 2 heteroatoms selected from N and S) arranged as a bicyclo [5,6] or [6,6] system. The W^5 heterocycle may be bonded to Y^2 through a carbon, nitrogen, sulfur or other atom by a stable covalent

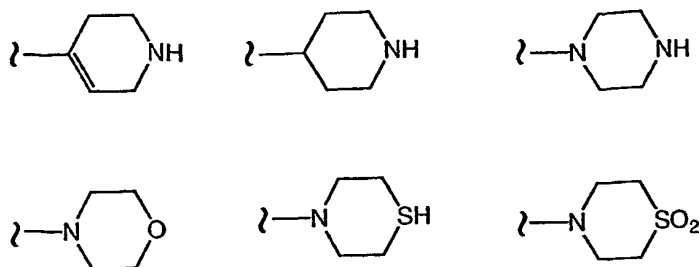
bond.

W⁵ heterocycles include for example, pyridyl, dihydropyridyl isomers, piperidine, pyridazinyl, pyrimidinyl, pyrazinyl, s-triazinyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, pyrazolyl, isothiazolyl, furanyl, thiofuranyl, thienyl, and pyrrolyl. W⁵ also includes, but is not limited to, examples such as:

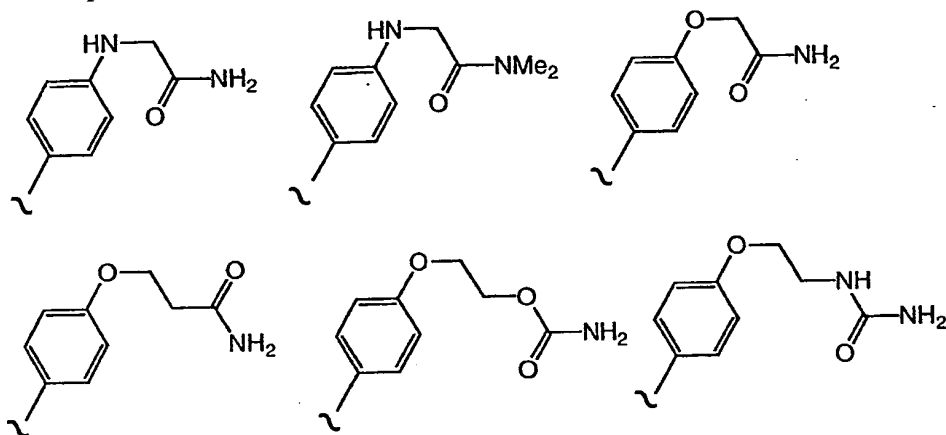


W⁵ carbocycles and heterocycles may be independently substituted with 0 to 3 R² groups, as defined above. For example, substituted W⁵ carbocycles include:





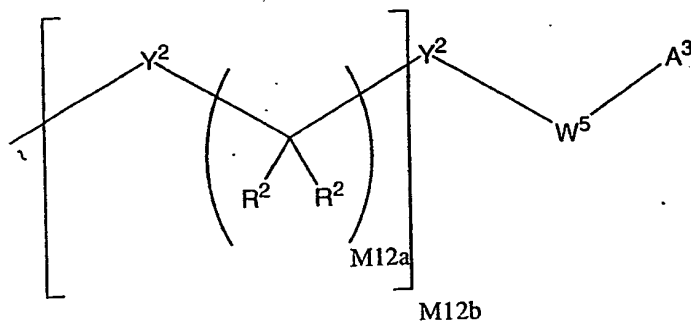
Examples of substituted phenyl carbocycles include:



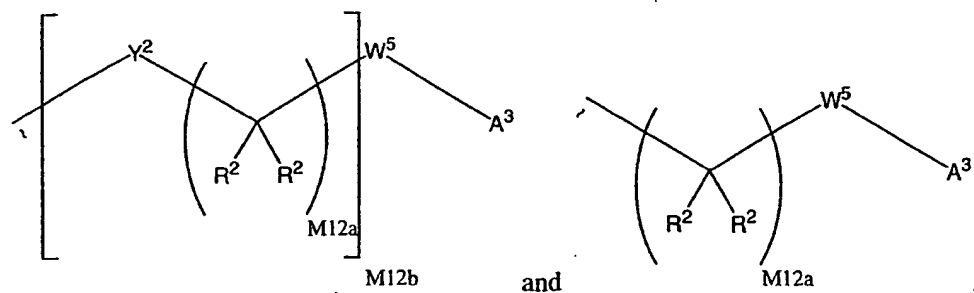
Embodiments

The following embodiments represent preferred choices for various substituents found on the candidate compounds of this invention. Each embodiment is to be construed as representing the enumerated substituent (or assembly of substituents) in combination with each and every other substituent that is not enumerated in the embodiment. For example, if W^3 is specified in an embodiment, then W^3 is locked but the remaining substituents can be set in any combination possible within the definition of A^3 .

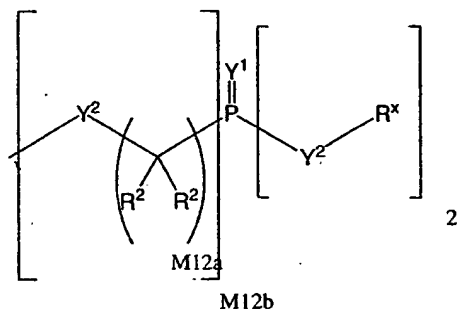
In an embodiment A^1 is



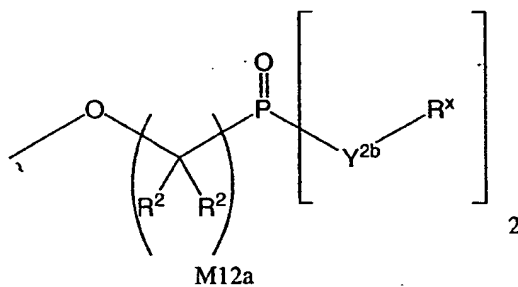
In an embodiment A^1 is



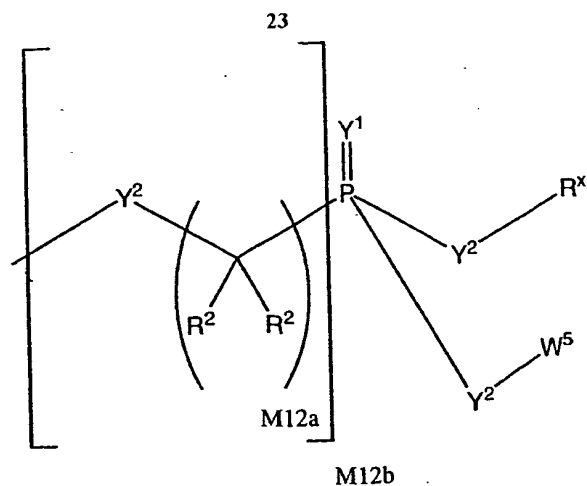
An embodiment of A^3 includes where M2 is 0, such as:



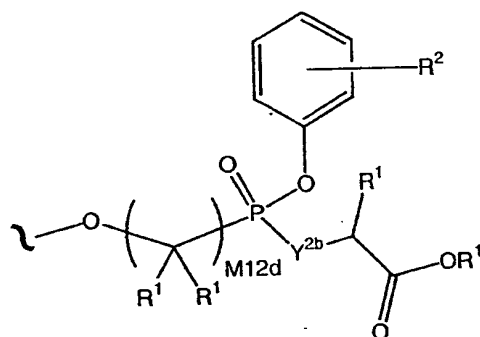
and where M12b is 1, Y^1 is oxygen, and Y^{2b} is oxygen (O) or nitrogen ($N(R^x)$) such as:



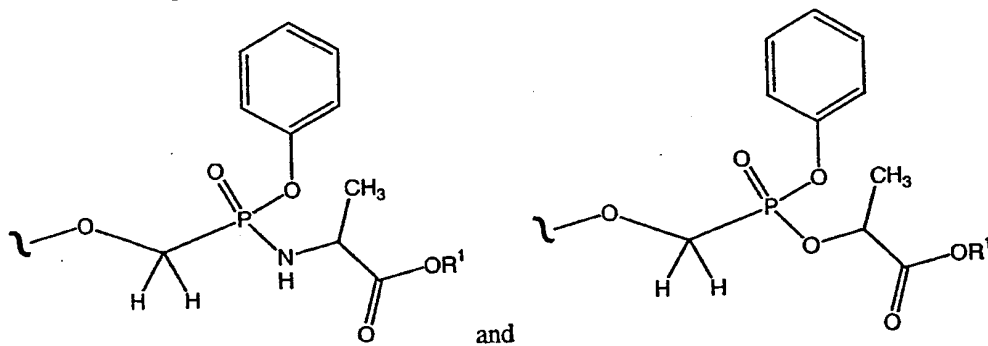
Another embodiment of A^3 is:



where W^5 is a carbocycle such as phenyl or substituted phenyl. Such embodiments include:

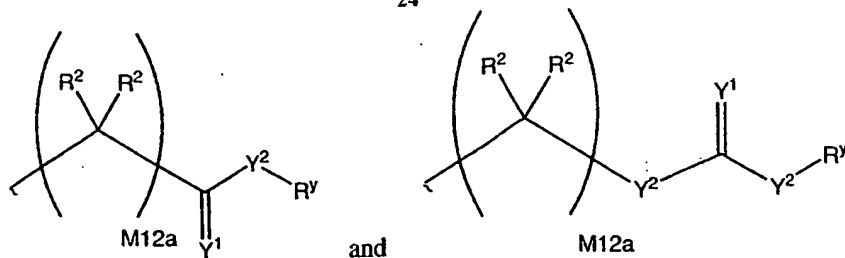


where Y^{2b} is O or $N(R^x)$; M12d is 1, 2, 3, 4, 5, 6, 7 or 8; and the phenyl carbocycle is substituted with 0 to 3 R^2 groups. Such embodiments of A^3 include phenyl phosphonamidate-alanate esters and phenyl phosphonate-lactate esters:

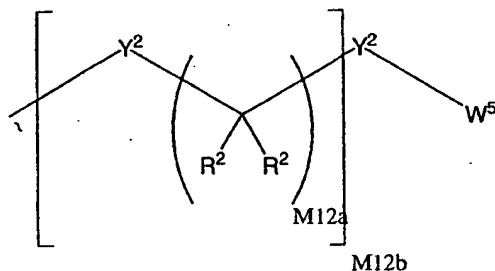


Embodiments of R^x include esters, carbamates, carbonates, thioesters, amides, thioamides, and urea groups:

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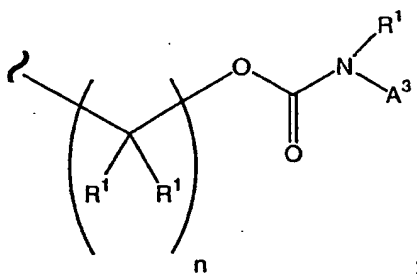
Embodiments of A² include where W³ is W⁵, such as:



Alternatively, A² is phenyl, substituted phenyl, benzyl, substituted benzyl, pyridyl or substituted pyridyl.

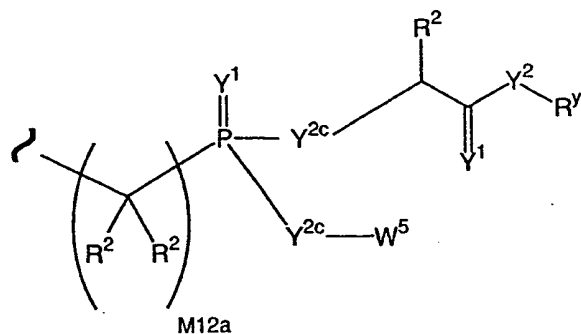
In other embodiments W⁴ may be R⁴, W^{5a} is a carbocycle or heterocycle and W^{5a} is optionally and independently substituted with 1, 2, or 3 R² groups. For example, W^{5a} may be 3,5-dichlorophenyl.

An embodiment of A¹ is:



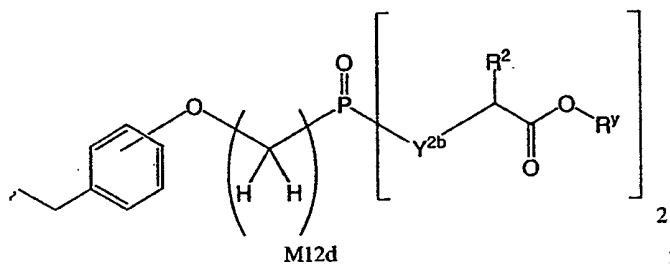
n is an integer from 1 to 18;

An embodiment of A³ optionally is of the formula:

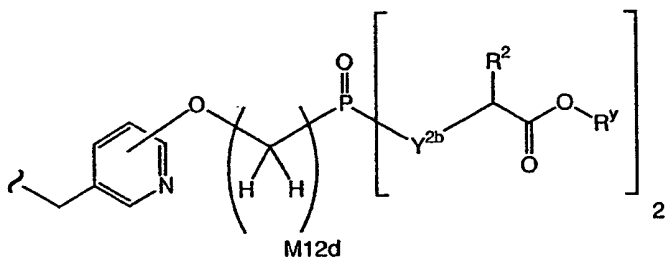


and Y^{2c} is O, $N(R^y)$ or S. For example, R^1 may be H and n may be 1.

An embodiment of A^1 optionally comprises a phosphonate group attached to an imidazole nitrogen through a heterocycle linker, such as:



and



where Y^{2b} is O or $N(R^2)$; and M12d is 1, 2, 3, 4, 5, 6, 7 or 8. The A^3 unit may be attached at any of the W^5 carbocycle or heterocycle ring atoms, e.g. ortho, meta, or para on a disubstituted W^5 .

A_1 optionally is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-W_3$, and W_3 is substituted with 1 to 3 A_3 groups.

A_2 optionally is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-W_3$.

A_3 optionally is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-P(Y_1)(Y_1R_{6a})(Y_1R_{6a})$.

X_2 and X_3 optionally are independently a bond, $-O-$, $-N(R_2)-$, $-N(OR_2)-$, $-N(N(R_2)(R_2))-$, $-S-$, $-SO-$, or $-SO_2-$.

Each Y_1 optionally is independently O, $N(R_2)$, $N(OR_2)$, or $N(N(R_2)(R_2))$, wherein each Y_1 is bound by two single bonds or one dot

R₁ optionally is independently H or alkyl of 1 to 12 carbon atoms.

R₂ optionally is independently H, R₃ or R₄ wherein each R₄ is independently substituted with 0 to 3 R₃ groups.

R₃ optionally is independently F, Cl, Br, I, -CN, N₃, -NO₂, -OR_{6a}, -OR₁, -N(R₁)₂, -N(R₁)(R_{6b}), -N(R_{6b})₂, -SR₁, -SR_{6a}, -S(O)R₁, -S(O)₂R₁, -S(O)OR₁, -S(O)OR_{6a}, -S(O)₂OR₁, -S(O)₂OR_{6a}, -C(O)OR₁, -C(O)R_{6c}, -C(O)OR_{6a}, -OC(O)R₁, -N(R₁)(C(O)R₁), -N(R_{6b})(C(O)R₁), -N(R₁)(C(O)OR₁), -N(R_{6b})(C(O)OR₁), -C(O)N(R₁)₂, -C(O)N(R_{6b})(R₁), -C(O)N(R_{6b})₂, -C(NR₁)(N(R₁)₂), -C(N(R_{6b}))(N(R₁)₂), -C(N(R₁))(N(R₁)(R_{6b})), -C(N(R_{6b}))(N(R₁)(R_{6b})), -C(N(R₁))(N(R_{6b})₂), -C(N(R_{6b}))(N(R_{6b})₂), -N(R₁)C(N(R₁))(N(R₁)₂), -N(R₁)C(N(R₁))(N(R₁)(R_{6b})), -N(R₁)C(N(R_{6b}))(N(R₁)₂), -N(R_{6b})C(N(R₁))(N(R₁)₂), -N(R_{6b})C(N(R_{6b}))(N(R₁)₂), -N(R_{6b})C(N(R₁))(N(R₁)(R_{6b})), -N(R₁)C(N(R_{6b}))(N(R₁)(R_{6b})), -N(R₁)C(N(R₁))(N(R_{6b})₂), -N(R_{6b})C(N(R_{6b}))(N(R₁)(R_{6b})), -N(R_{6b})C(N(R₁))(N(R_{6b})₂), -N(R₁)C(N(R_{6b}))(N(R_{6b})₂), -N(R_{6b})C(N(R_{6b}))(N(R_{6b})₂), =O, =S, =N(R₁), =N(R_{6b}) or W₅.

R₄ optionally is independently alkyl of 1 to 12 carbon atoms, alkenyl of 2 to 12 carbon atoms, or alkynyl of 2 to 12 carbon atoms.

R₅ optionally is independently R₄ wherein each R₄ is substituted with 0 to 3 R₃ groups; or R₅ is independently alkylene of 1 to 12 carbon atoms, alkenylene of 2 to 12 carbon atoms, or alkynylene of 2-12 carbon atoms any one of which alkylene, alkenylene or alkynylene is substituted with 0-3 R₃ groups.

R_{6a} is independently H or an ether- or ester-forming group.

R_{6b} is independently H, a protecting group for amino or the residue of a carboxyl-containing compound.

R_{6c} is independently H or the residue of an amino-containing compound.

W₄ is R₅, -C(Y₁)R₅, -C(Y₁)W₅, -SO₂R₅, or -SO₂W₅.

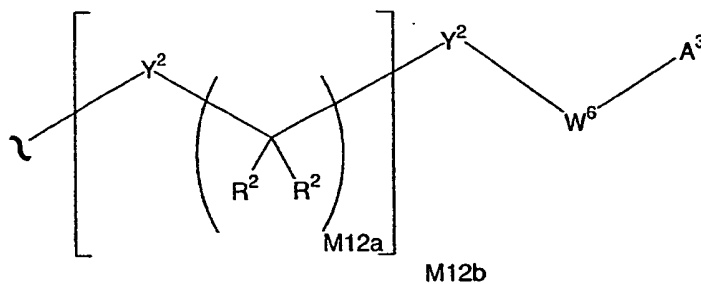
W₅ is carbocycle or heterocycle wherein W₅ is independently substituted with 0 to 3 R₂ groups.

m₁ is independently an integer from 0 to 12, wherein the sum of all m₁'s within each individual embodiment of A₁, A₂ or A₃ is 12 or less.

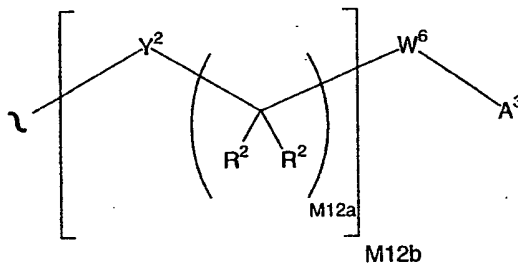
m₂ is independently an integer from 0 to 2.

In another embodiment A^1 is $-(C(R_2)(R_2))_{m1}-W_3$, wherein W_3 is substituted with 1 A_3 group, A_2 is $-(C(R_2)(R_2))_{m1}-W_3$, and A_3 is $-(C(R_2)(R_2))_{m1}P(Y_1)(Y_1R_{6a})(Y_1R_{6a})$.

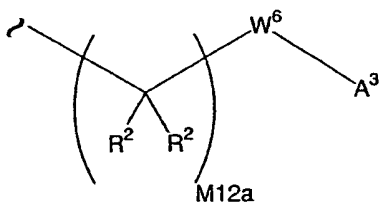
In an embodiment A^1 is of the formula:



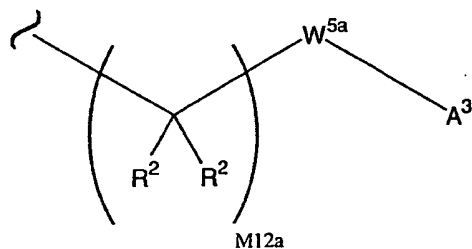
In an embodiment A^1 is of the formula:



In an embodiment A^1 is of the formula:



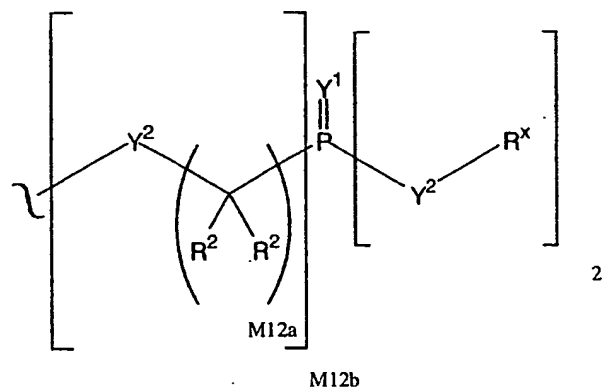
In an embodiment A^1 is of the formula:



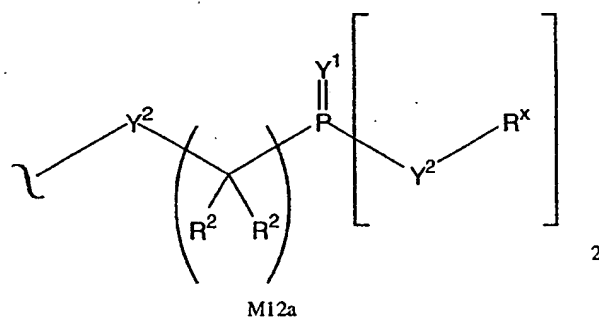
and W^{5a} is a carbocycle or a heterocycle where W^{5a} is independently substituted with 0 or 1 R^2 groups.

In an embodiment M12a is 1.

In an embodiment A³ is of the formula:

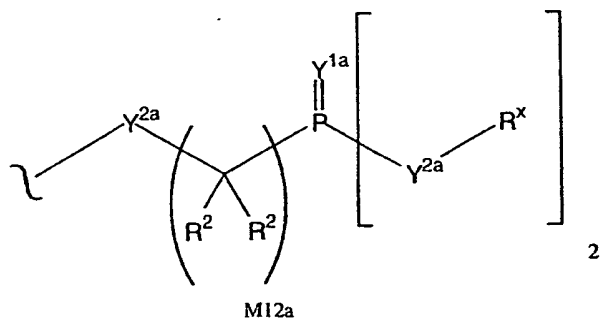


In an embodiment A³ is of the formula:



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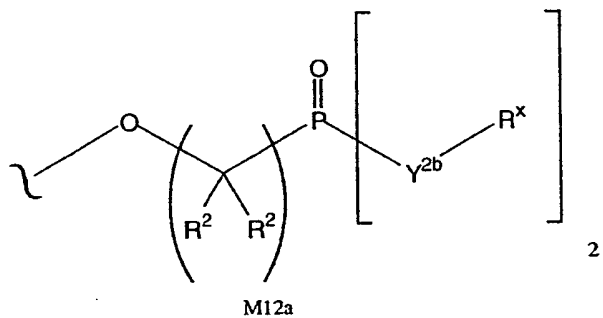
In an embodiment A³ is of the formula:



Y^{1a} is O or S; and

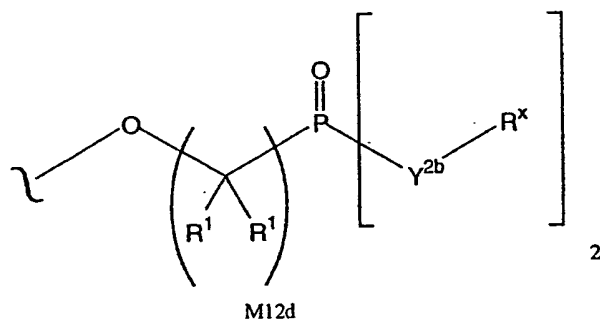
Y^{2a} is O, N(R^x) or S.

In an embodiment A³ is of the formula:



and Y^{2b} is O or N(R^x).

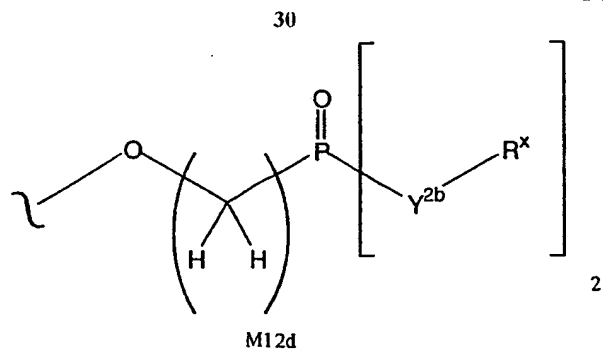
In an embodiment A³ is of the formula:



Y^{2b} is O or N(R^x); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In and embodiment A³ is of the formula:

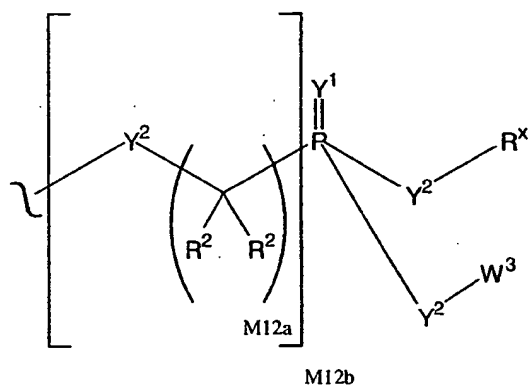


Y^{2b} is O or N(R^x); and

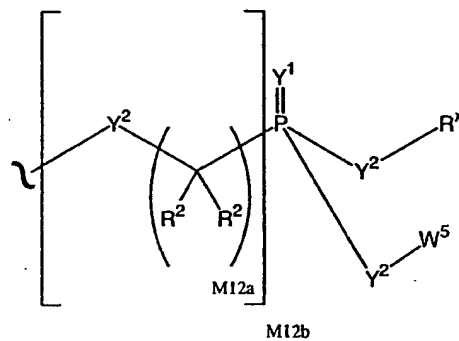
M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment M12d is 1.

In an embodiment A³ is of the formula:

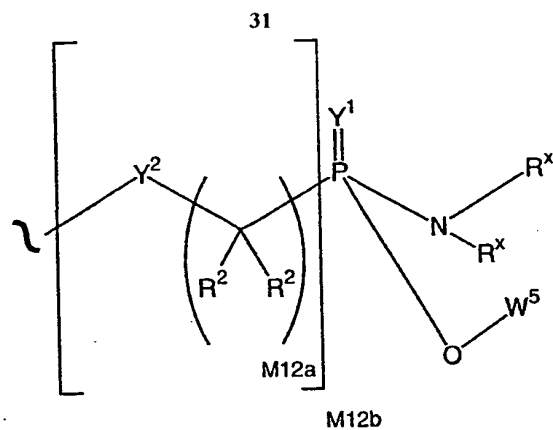


In an embodiment A³ is of the formula:



In an embodiment W³ is a carbocycle.

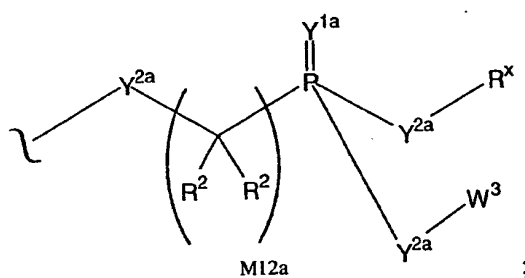
In an embodiment A³ is of the formula:



In an embodiment W^5 is phenyl.

In an embodiment M12b is 1.

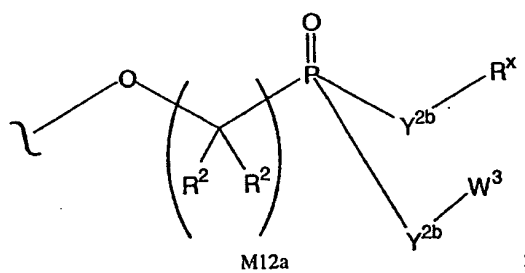
In an embodiment A^3 is of the formula:



Y^{1a} is O or S; and

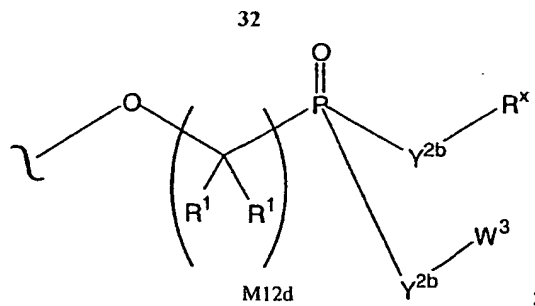
Y^{2a} is O, $N(R^x)$ or S.

In an embodiment A^3 is of the formula:



and Y^{2b} is O or $N(R^x)$.

In an embodiment A^3 is of the formula:



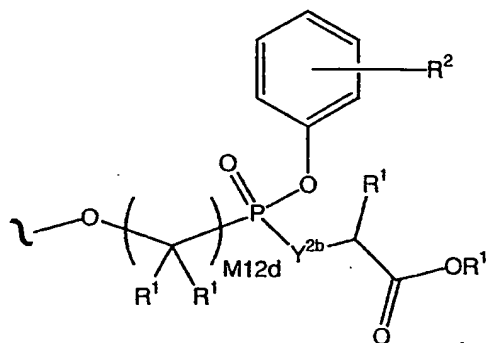
Y^{2b} is O or N(R^x); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment R¹ is H.

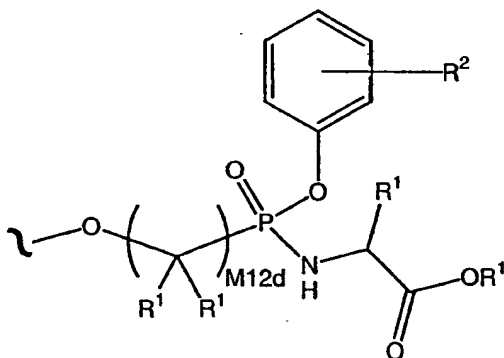
In an embodiment M12d is 1.

In an embodiment A³ is of the formula:

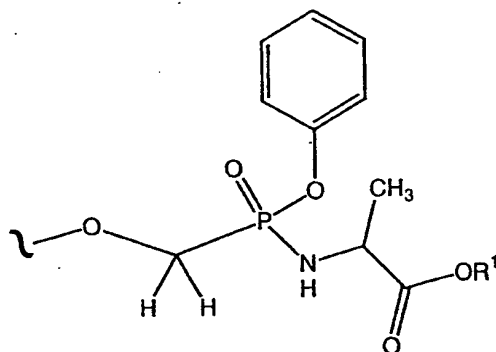
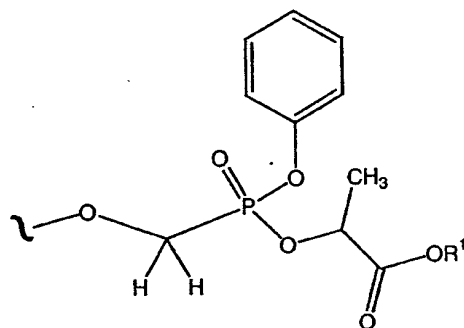
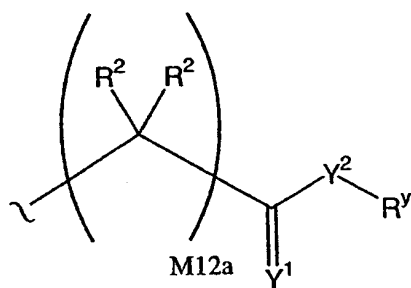
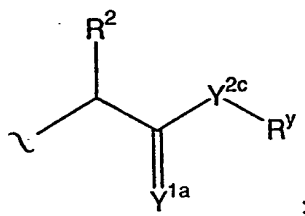


wherein the phenyl carbocycle is substituted with 0 to 3 R² groups.

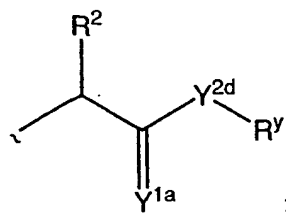
In an embodiment A³ is of the formula:



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In an embodiment A³ is of the formula:In an embodiment A³ is of the formula:In an embodiment R^x is of the formula:In an embodiment R^x is of the formula:Y^{1a} is O or S; andY^{2c} is O, N(R^y) or S.

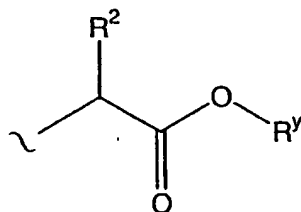
In an embodiment R^x is of the formula:



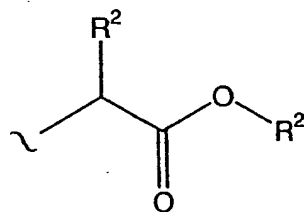
Y^{1a} is O or S; and

Y^{2d} is O or $\text{N}(\text{R}^y)$.

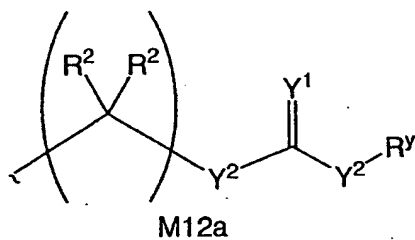
In an embodiment R^x is of the formula:



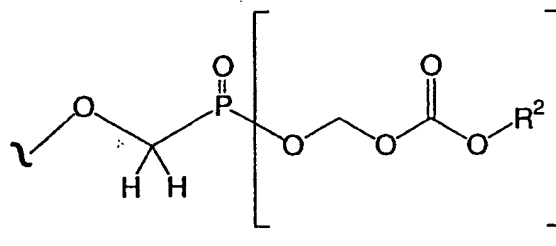
In an embodiment R^x is of the formula:



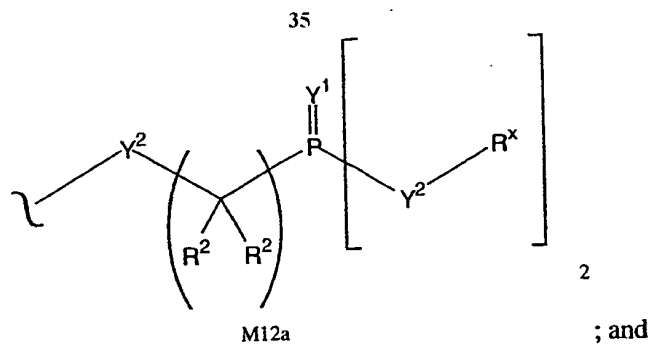
In an embodiment R^x is of the formula:



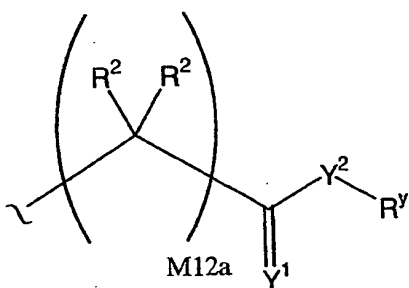
In an embodiment A^3 is of the formula:



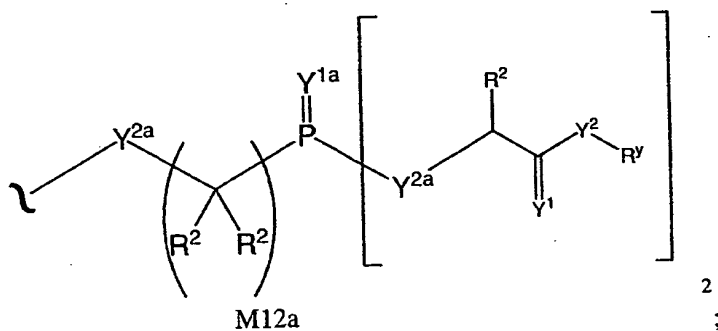
In an embodiment A^3 is of the formula:



R^x is of the formula:



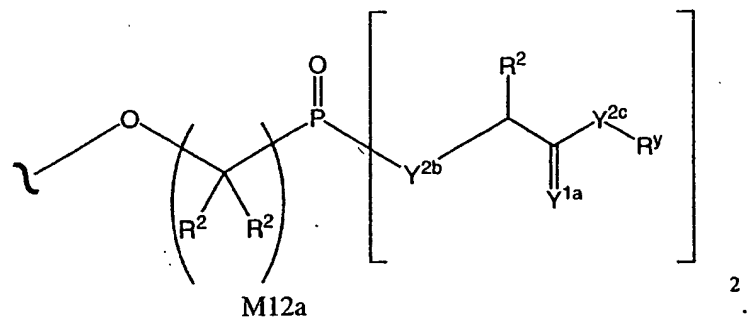
In an embodiment A³ is of the formula:



Y^{1a} is O or S; and

Y^{2a} is O, N(R²) or S.

In an embodiment A³ is of the formula:

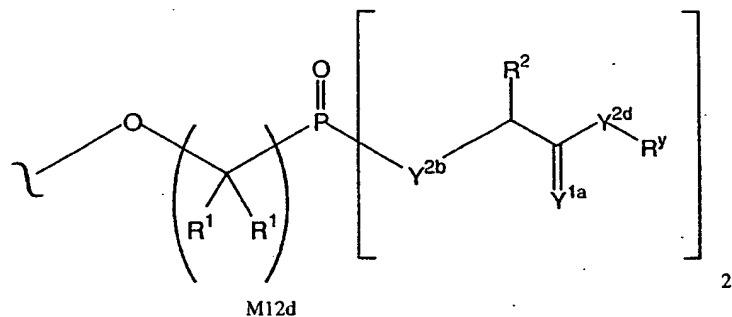


Y^{1a} is O or S;

Y^{2b} is O or N(R²); and

Y^{2c} is O, N(R^y) or S.

In an embodiment A³ is of the formula:



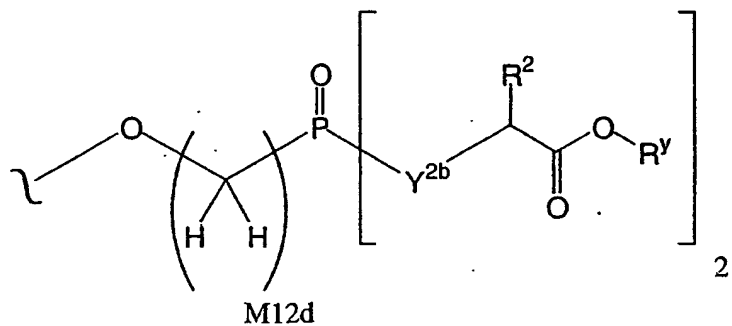
Y^{1a} is O or S;

Y^{2b} is O or N(R²);

Y^{2d} is O or N(R^y); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

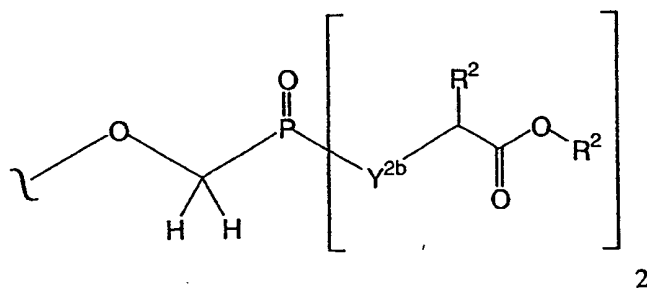
In an embodiment A³ is of the formula:



Y^{2b} is O or $N(R^2)$; and

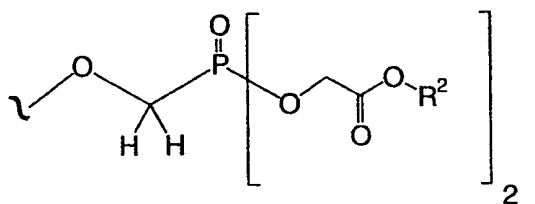
M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A^3 is of the formula:

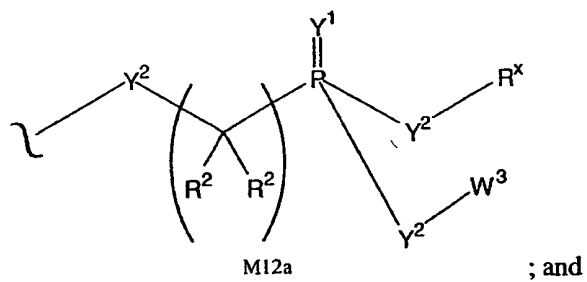


and Y^{2b} is O or $N(\mathbb{R}^2)$.

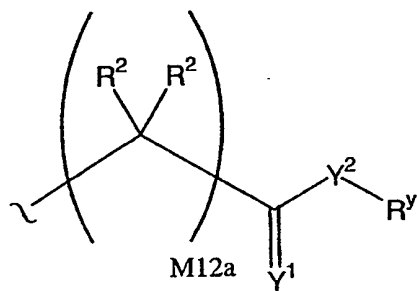
In an embodiment A^3 is of the formula:



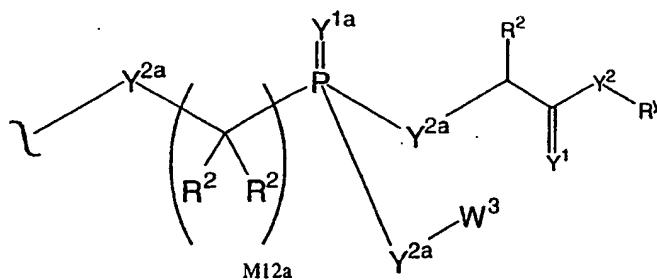
In an embodiment A^3 is of the formula:



R^x is of the formula:



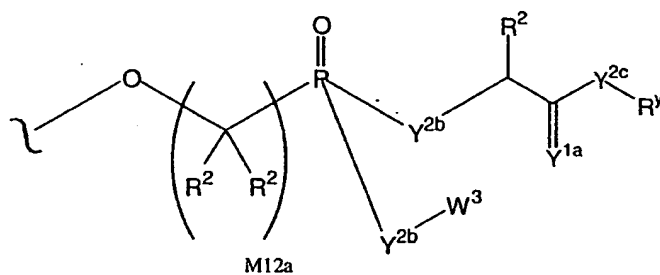
In an embodiment A^3 is of the formula:



Y^{1a} is O or S; and

Y^{2a} is O, N(R^2) or S.

In an embodiment A^3 is of the formula:

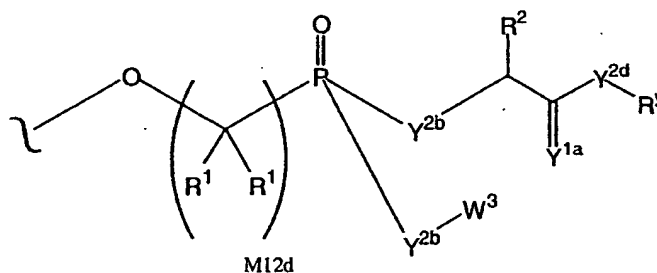


Y^{1a} is O or S;

Y^{2b} is O or N(R^2); and

Y^{2c} is O, N(R^y) or S.

In an embodiment A^3 is of the formula:



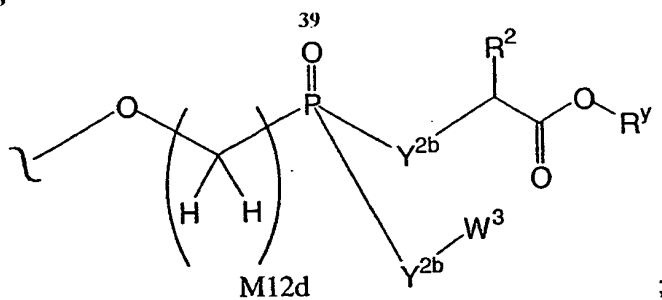
Y^{1a} is O or S;

Y^{2b} is O or N(R^2);

Y^{2d} is O or N(R^y); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

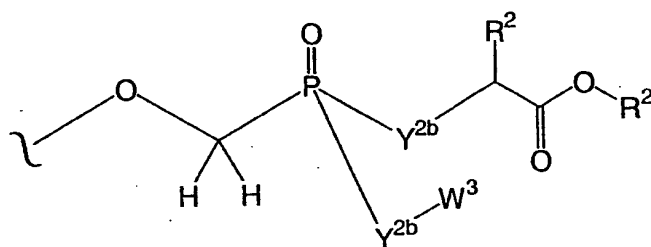
In an embodiment A^3 is of the formula:



Y^{2b} is O or $N(R^2)$; and

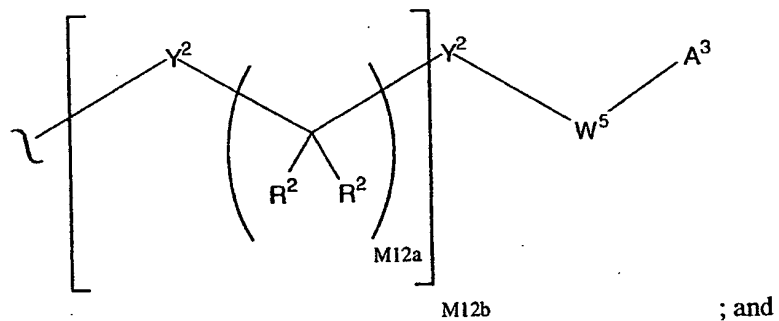
M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A^3 is of the formula:

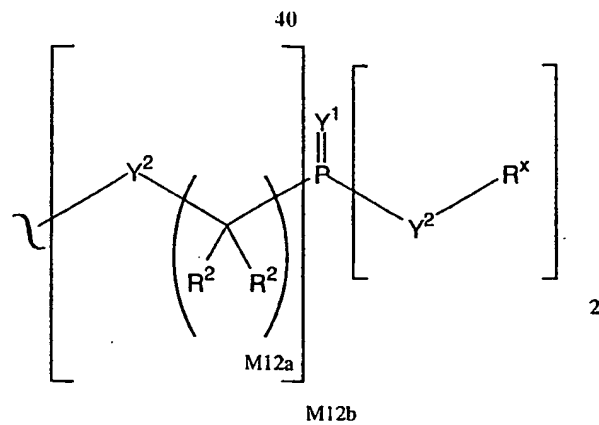


and Y^{2b} is O or $N(R^2)$.

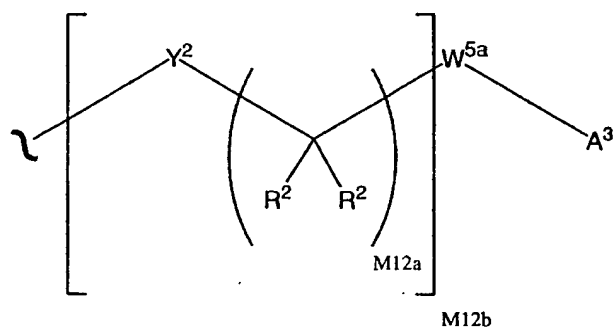
In an embodiment A^1 is of the formula:



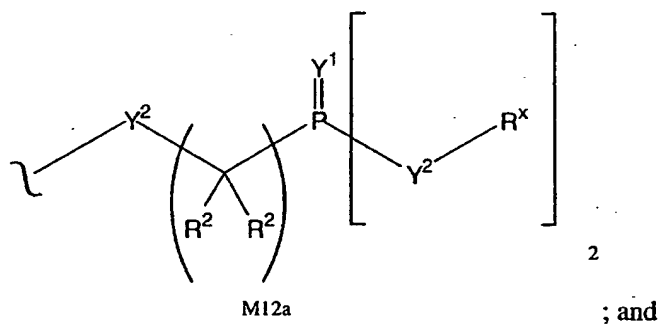
A^3 is of the formula:



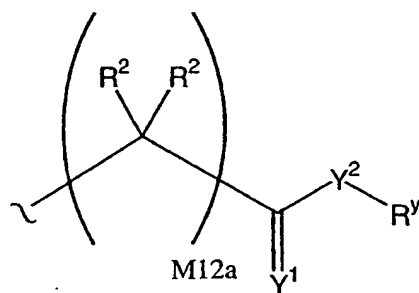
In an embodiment A¹ is of the formula:



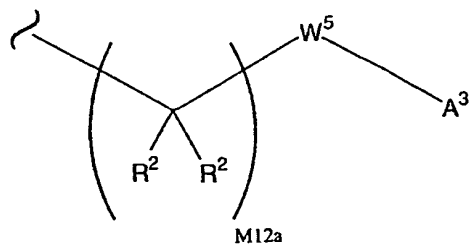
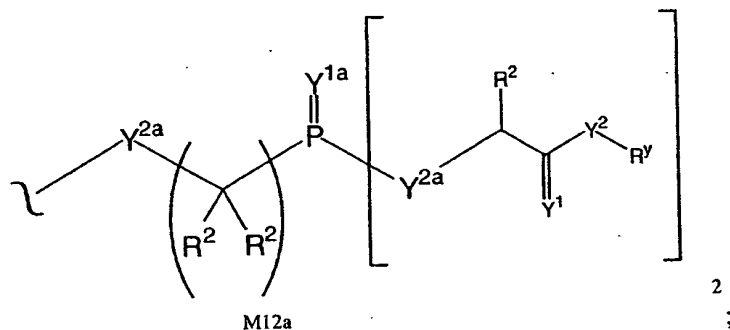
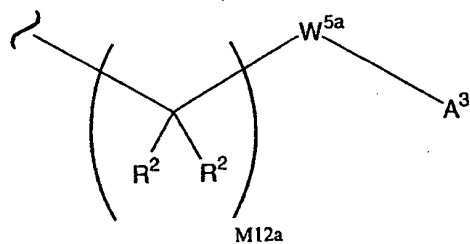
A³ is of the formula:

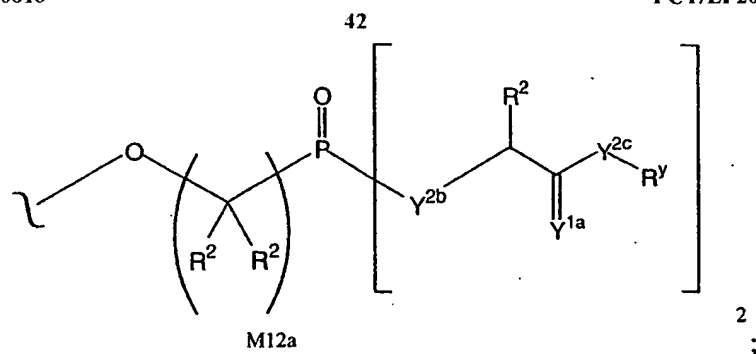


Rˣ is of the formula:



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In an embodiment A¹ is of the formula:A³ is of the formula:Y^{1a} is O or S; andY^{2a} is O, N(R²) or S.In an embodiment A¹ is of the formula:W^{5a} is a carbocycle independently substituted with 0 or 1 R² groups;A³ is of the formula:

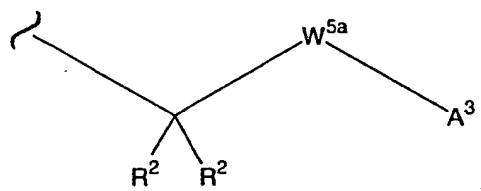


Y^{1a} is O or S;

Y^{2b} is O or $N(R^2)$; and

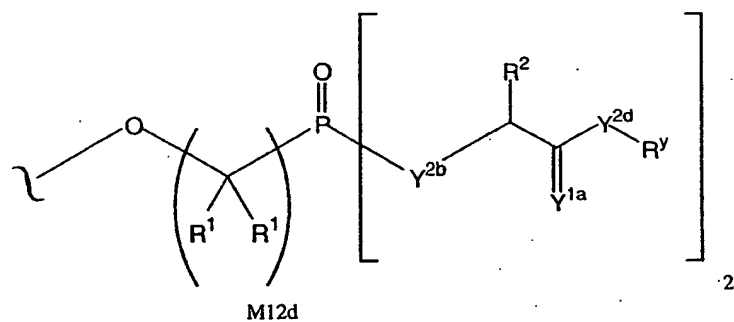
Y^{2c} is O, $N(R^y)$ or S.

In an embodiment A^1 is of the formula:



W^{5a} is a carbocycle independently substituted with 0 or 1 R^2 groups;

A^3 is of the formula:



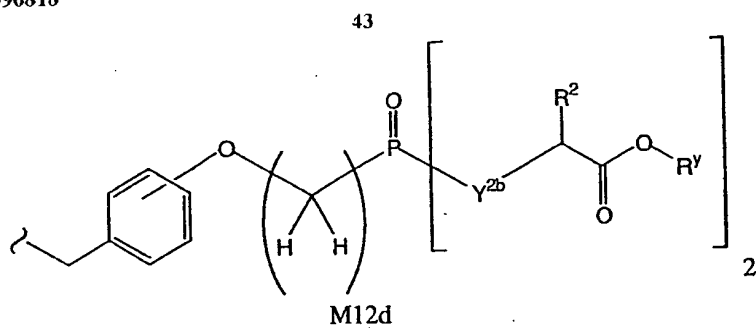
Y^{1a} is O or S;

Y^{2b} is O or $N(R^2)$;

Y^{2d} is O or $N(R^y)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

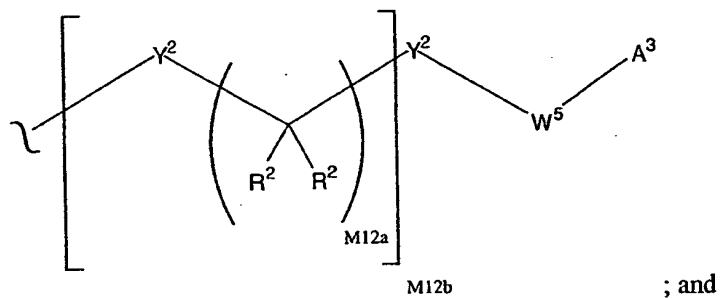
In an embodiment A^1 is of the formula:



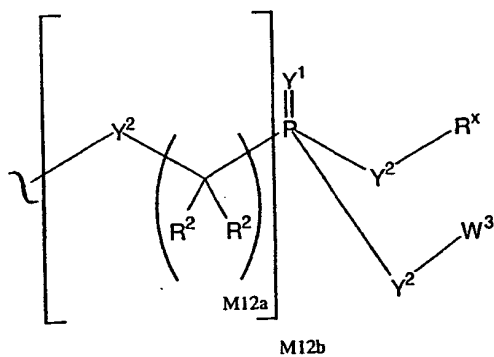
Y^{2b} is O or $N(R^2)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

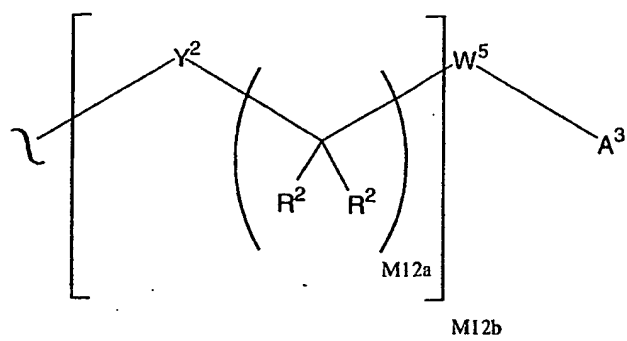
In an embodiment A^1 is of the formula:



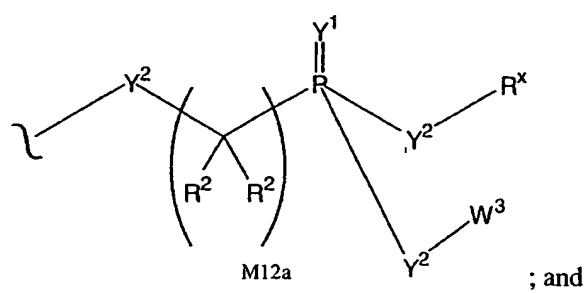
A^3 is of the formula:



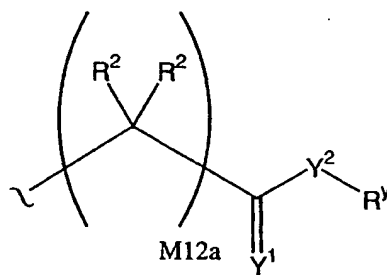
In an embodiment A^1 is of the formula:



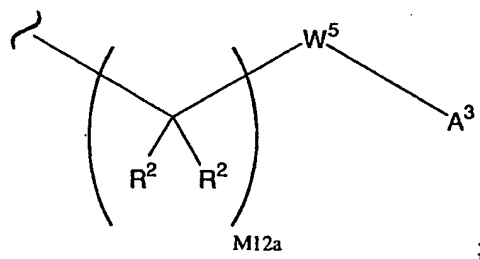
A³ is of the formula:



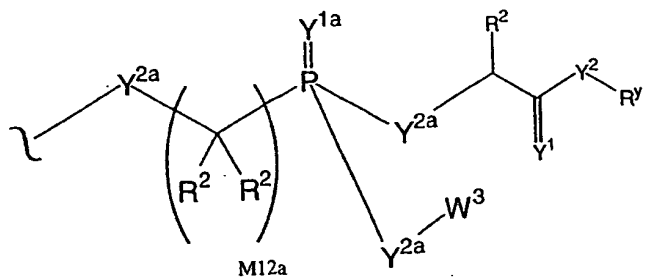
R^x is of the formula:



In an embodiment A¹ is of the formula:



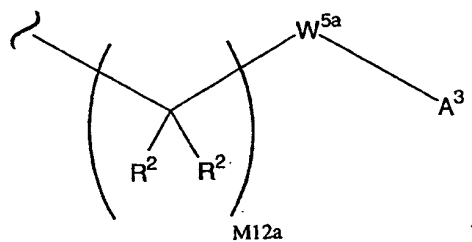
A³ is of the formula:



Y^{1a} is O or S; and

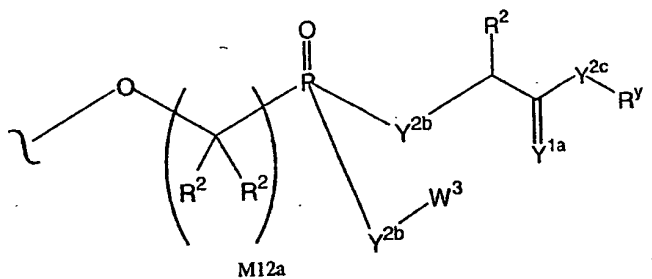
Y^{2a} is O, N(R²) or S.

In an embodiment A¹ is of the formula:



W^{5a} is a carbocycle independently substituted with 0 or 1 R² groups;

A³ is of the formula:

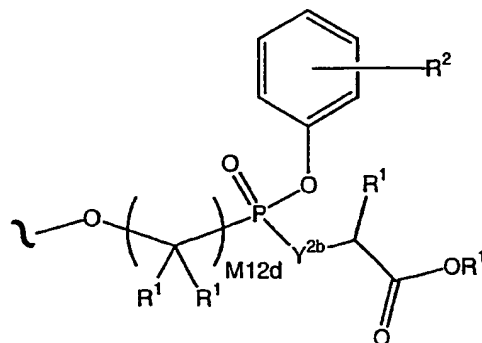


Y^{1a} is O or S;

Y^{2b} is O or N(R²); and

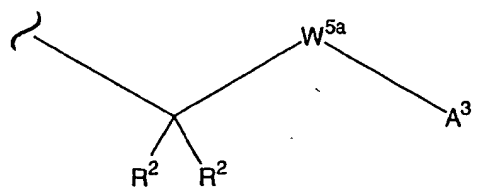
Y^{2c} is O, N(R^y) or S.

In an embodiment A^3 is of the formula:



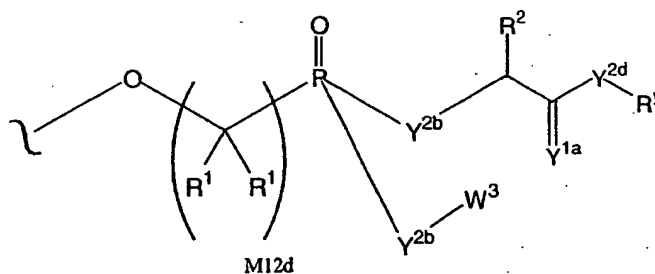
wherein the phenyl carbocycle is substituted with 0 to 3 R^2 groups.

In an embodiment A^1 is of the formula:



W^{5a} is a carbocycle or heterocycle where W^{5a} is independently substituted with 0 or 1 R^2 groups;

A^3 is of the formula:



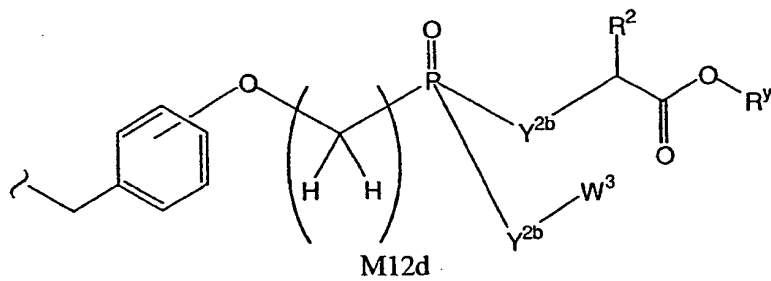
Y^{1a} is O or S;

Y^{2b} is O or $N(R^2)$;

Y^{2d} is O or $N(R^y)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

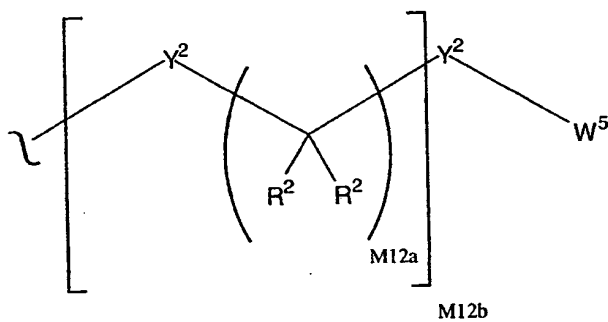
In an embodiment A¹ is of the formula:



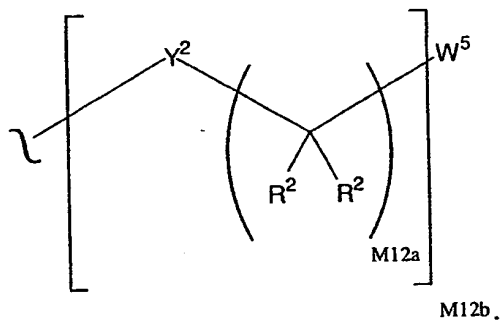
Y^{2b} is O or N(R²); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A² is of the formula:



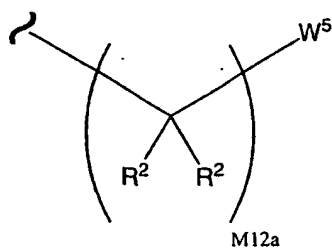
In an embodiment A² is of the formula:



In an embodiment M12b is 1.

In an embodiment M12b is 0, Y² is a bond and W⁵ is a carbocycle or heterocycle where W⁵ is optionally and independently substituted with 1, 2, or 3 R² groups.

In an embodiment A^2 is of the formula:

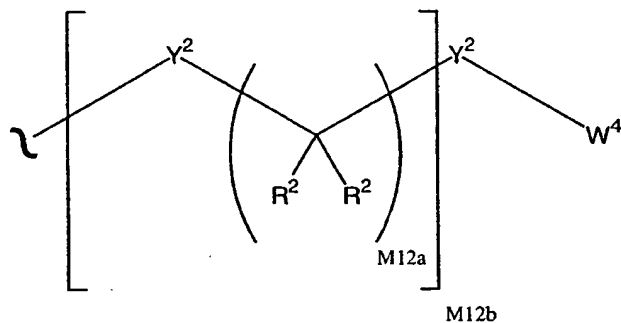


and W^{5a} is a carbocycle or heterocycle where W^{5a} is optionally and independently substituted with 1, 2, or 3 R^2 groups.

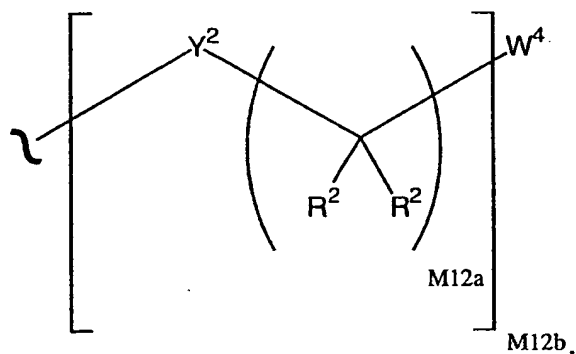
In an embodiment M12a is 1.

In an embodiment A^2 is selected from phenyl, substituted phenyl, benzyl, substituted benzyl, pyridyl and substituted pyridyl.

In an embodiment A^2 is of the formula:

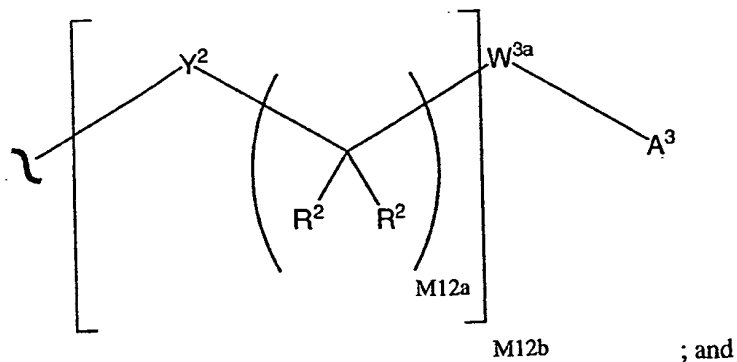


In an embodiment A^2 is of the formula:



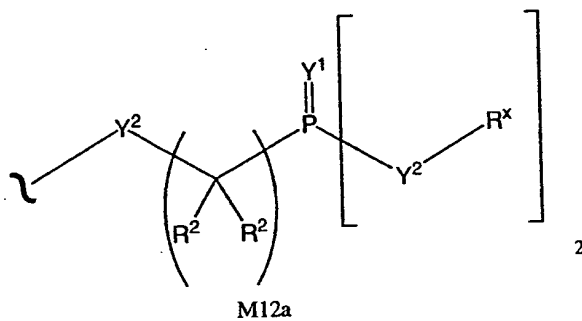
In an embodiment M12b is 1.

In an embodiment A^1 is of the formula:

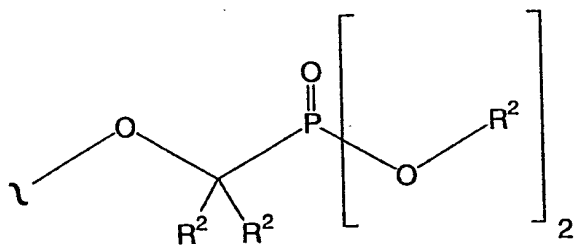


; and

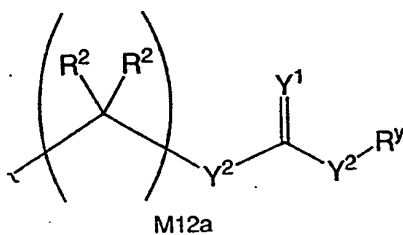
A^3 is of the formula:



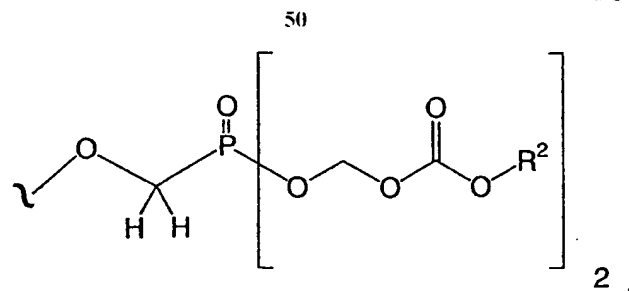
In an embodiment A^3 is of the formula:



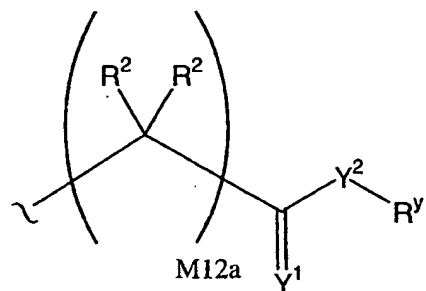
In an embodiment R^x is of the formula:



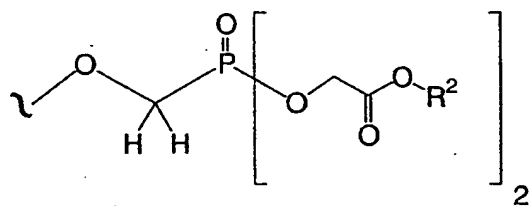
In an embodiment A^3 is of the formula:



In an embodiment R^x is of the formula:

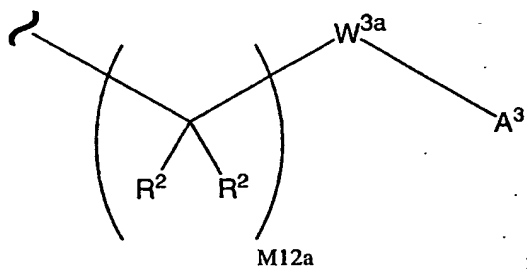


In an embodiment A^3 is of the formula:

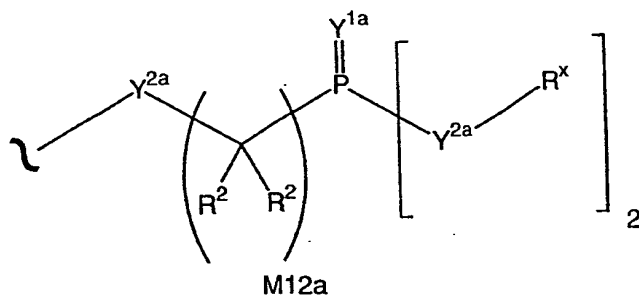


In an embodiment R^4 is isopropyl.

In an embodiment A^1 is of the formula:

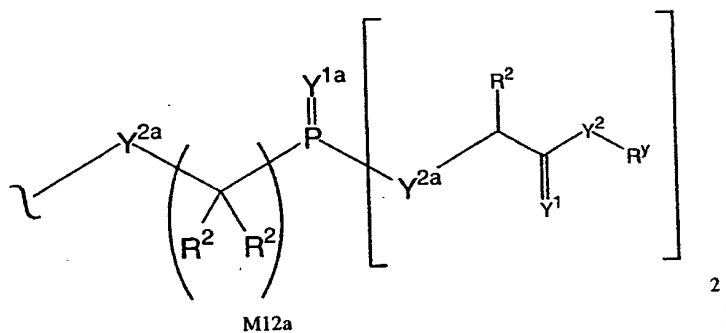


A³ is of the formula:



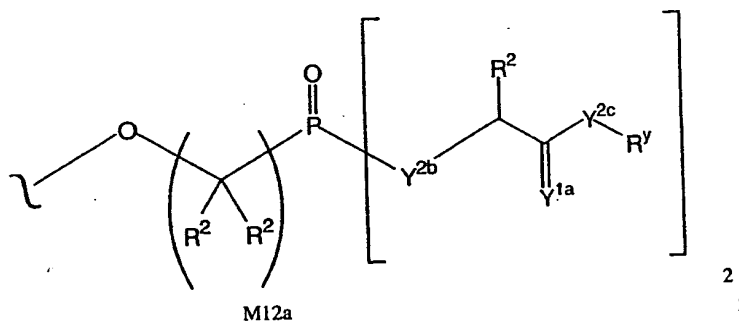
and Y^{1a} is O or S.

In an embodiment A³ is of the formula:



and Y^{2a} is O, N(R²) or S.

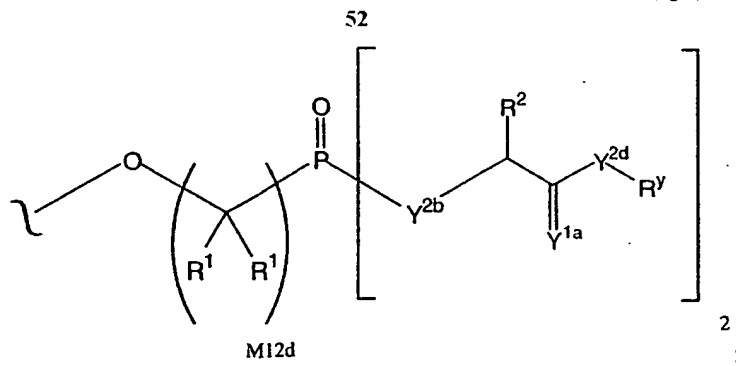
In an embodiment A³ is of the formula:



Y^{2b} is O or N(R²); and

Y^{2c} is O, N(R^ʸ) or S.

In an embodiment A³ is of the formula:



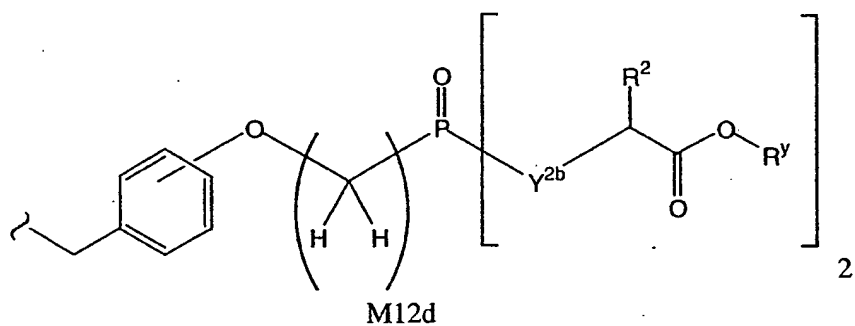
Y^{1a} is O or S;

Y^{2b} is O or $N(R^2)$;

Y^{2d} is O or $N(R^y)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

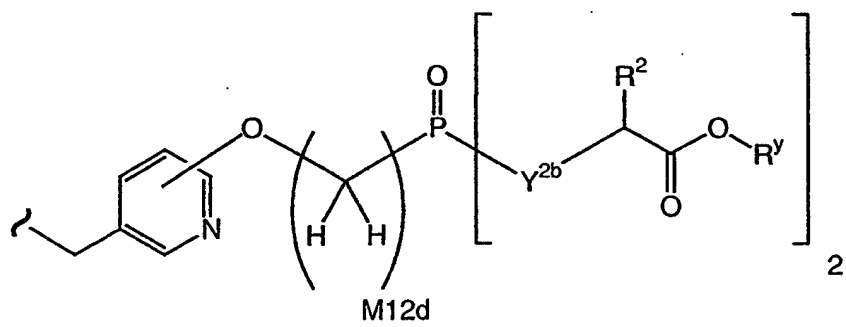
In an embodiment A¹ is of the formula:



Y^{2b} is O or $N(R^2)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

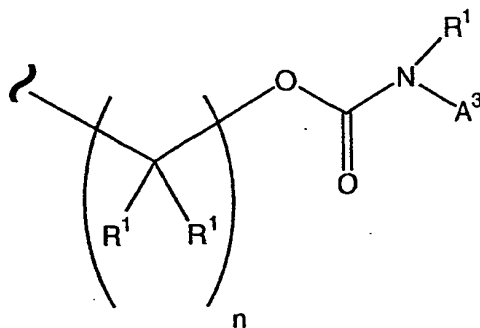
In an embodiment A¹ is of the formula:



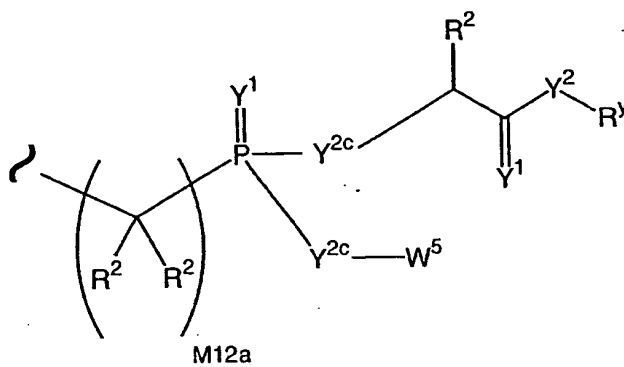
and Y^{2b} is O or $N(R^2)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A^1 is of the formula:



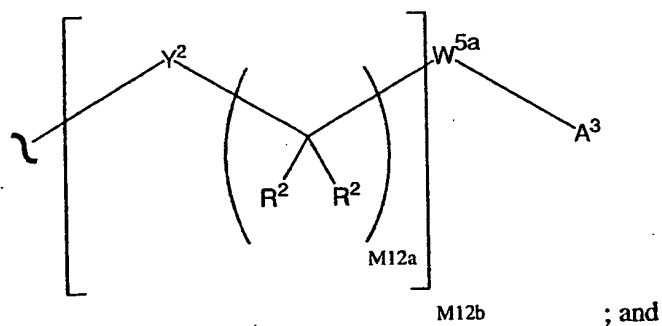
n is an integer from 1 to 18; A^3 is of the formula:



and Y^{2c} is O, $N(R^y)$ or S.

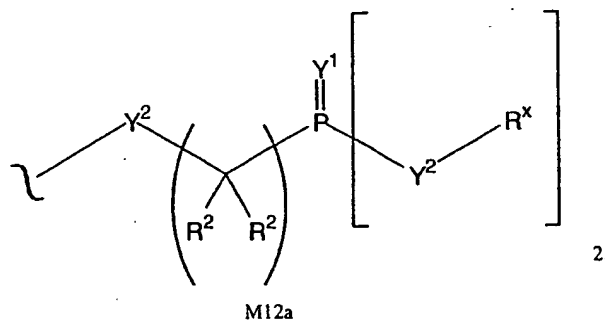
In an embodiment R^1 is H and n is 1.

In an embodiment A^1 is of the formula:

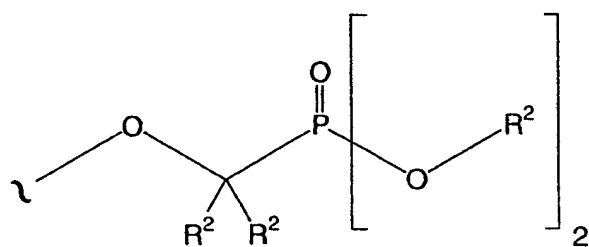


A^3 is of the formula:

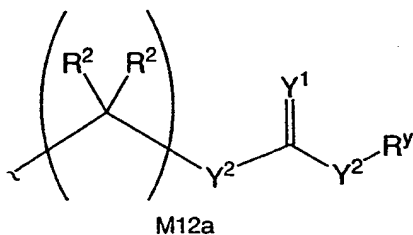
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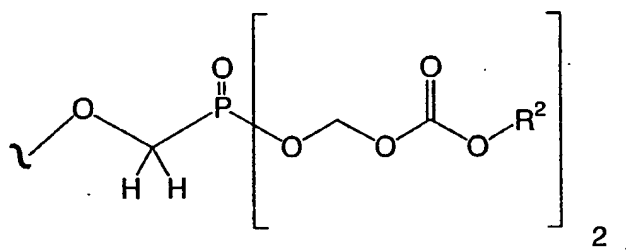
In an embodiment A³ is of the formula:



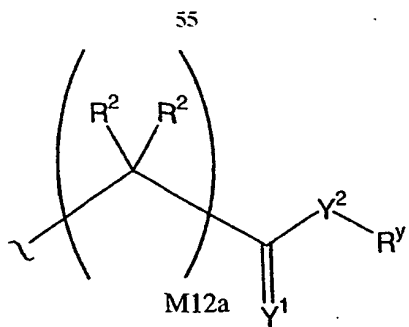
In an embodiment R^x is of the formula:



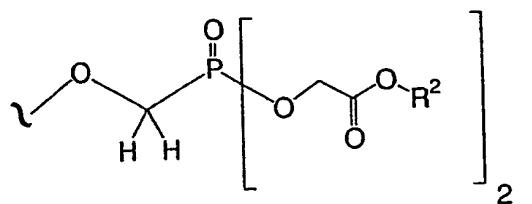
In an embodiment A³ is of the formula:



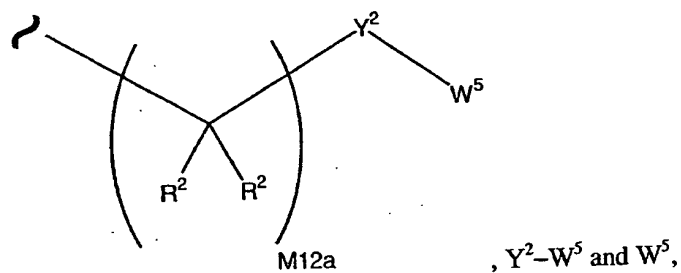
In an embodiment R^x is of the formula:



In an embodiment A³ is of the formula:

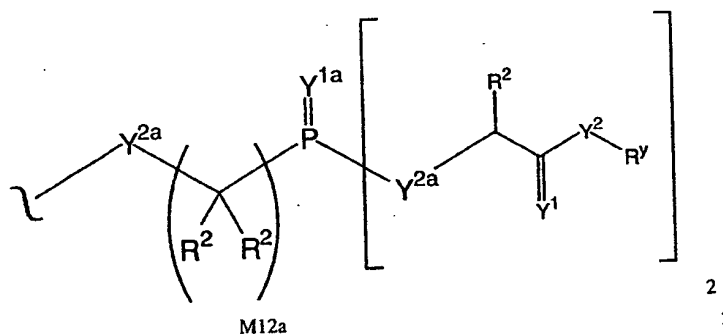


In an embodiment A² is selected from:



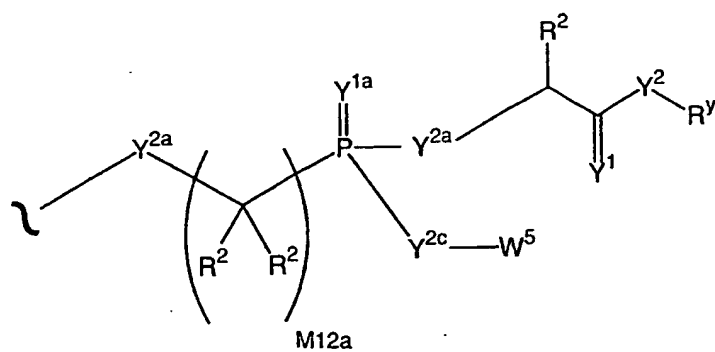
where W⁵ is a carbocycle or a heterocycle and where W⁵ is independently substituted with 0 to 3 R² groups.

In an embodiment A³ is of the formula:



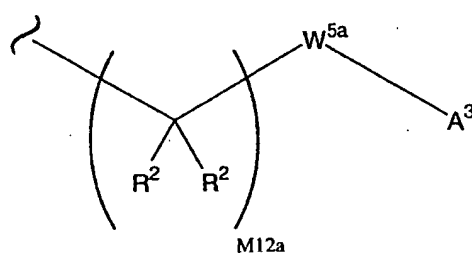
and Y^{2a} is O, N(R²) or S.

In an embodiment A³ is of the formula:

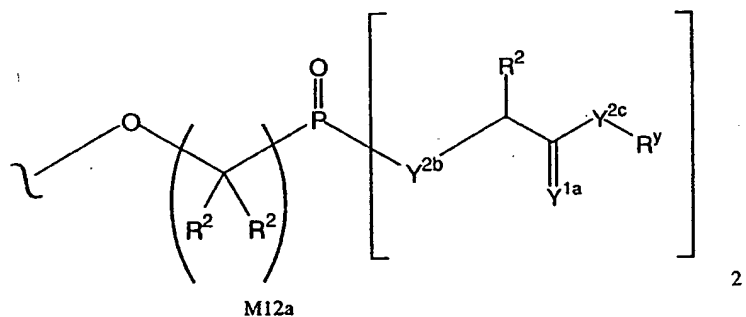


and Y^{2c} is O, $N(R^y)$ or S.

In an embodiment A^1 is of the formula:



A^3 is of the formula:

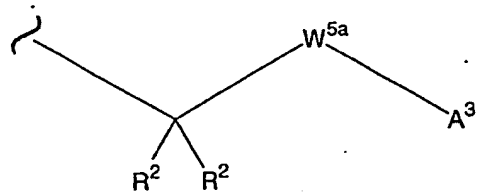


W^{5a} is a carbocycle or a heterocycle where the carbocycle or heterocycle is independently substituted with 0 to 3 R^2 groups;

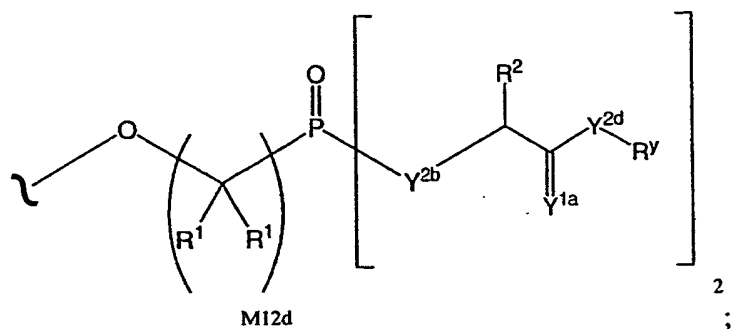
Y^{2b} is O or $N(R^2)$; and

Y^{2c} is O, $N(R^y)$ or S.

In an embodiment A^1 is of the formula:



A³ is of the formula:



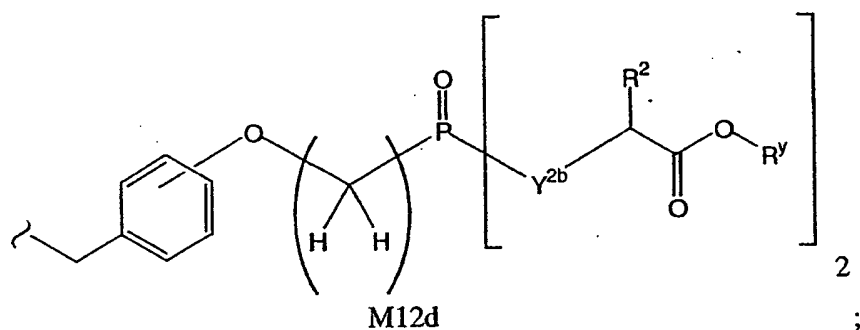
Y^{1a} is O or S;

Y^{2b} is O or N(R²);

Y^{2d} is O or N(R^y); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A¹ is of the formula:

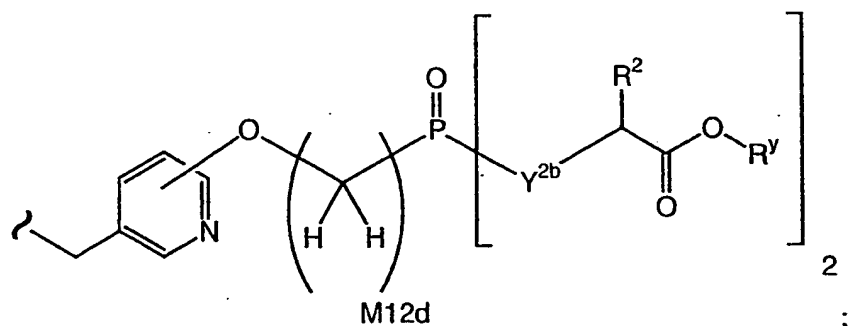


Y^{2b} is O or N(R²); and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A¹ is of the formula:

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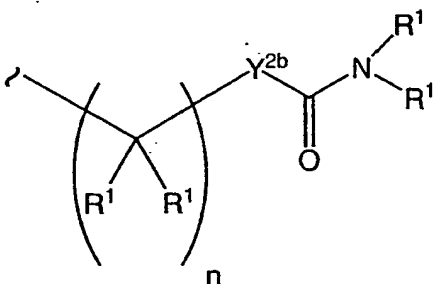


and Y^{2b} is O or $N(R^2)$; and

M12d is 1, 2, 3, 4, 5, 6, 7 or 8.

In an embodiment A^2 is a phenyl substituted with 0 to 3 R^2 groups.

In an embodiment W^4 is of the formula:



wherein n is an integer from 1 to 18; and Y^{2b} is O or $N(R^2)$.

In an embodiment

A_1 is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-W_3$, wherein W_3 is substituted with 1 to 3 A_3 groups;

A_2 is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-W_3$;

A_3 is $-(X_2-(C(R_2)(R_2))_{m1}-X_3)_{m1}-P(Y_1)(Y_1R_{6a})(Y_1R_{6a})$;

X_2 and X_3 are independently a bond, -O-, - $N(R_2)$ -, - $N(OR_2)$ -, - $N(N(R_2)(R_2))$ -, -S-, -SO-, or -SO₂-;

each Y_1 is independently O, $N(R_2)$, $N(OR_2)$, or $N(N(R_2)(R_2))$, wherein each Y_1 is bound by two single bonds or one double bond;

R_1 is independently H or alkyl of 1 to 12 carbon atoms;

R_2 is independently H, R_3 or R_4 wherein each R_4 is independently substituted with 0 to 3 R_3 groups;

R₃ is independently F, Cl, Br, I, -CN, N₃, -NO₂, -OR_{6a}, -OR₁, -N(R₁)₂, -N(R₁)(R_{6b}), -N(R_{6b})₂, -SR₁, -SR_{6a}, -S(O)R₁, -S(O)₂R₁, -S(O)OR₁, -S(O)OR_{6a}, -S(O)₂OR₁, -S(O)₂OR_{6a}, -C(O)OR₁, -C(O)R_{6c}, -C(O)OR_{6a}, -OC(O)R₁, -N(R₁)(C(O)R₁), -N(R_{6b})(C(O)R₁), -N(R₁)(C(O)OR₁), -N(R_{6b})(C(O)OR₁), -C(O)N(R₁)₂, -C(O)N(R_{6b})(R₁), -C(O)N(R_{6b})₂, -C(NR₁)(N(R₁)₂), -C(N(R_{6b}))(N(R₁)₂), -C(N(R₁))(N(R₁)(R_{6b})), -C(N(R_{6b}))(N(R₁)(R_{6b})), -C(N(R₁))(N(R_{6b})₂), -C(N(R_{6b}))(N(R_{6b})₂), -N(R₁)C(N(R₁))(N(R₁)₂), -N(R₁)C(N(R₁))(N(R₁)(R_{6b})), -N(R₁)C(N(R_{6b}))(N(R₁)₂), -N(R_{6b})C(N(R₁))(N(R₁)₂), -N(R_{6b})C(N(R_{6b}))(N(R₁)₂), -N(R_{6b})C(N(R₁))(N(R₁)(R_{6b})), -N(R₁)C(N(R_{6b}))(N(R₁)(R_{6b})), -N(R₁)C(N(R₁))(N(R_{6b})₂), -N(R_{6b})C(N(R_{6b}))(N(R₁)(R_{6b})), -N(R_{6b})C(N(R₁))(N(R_{6b})₂), -N(R₁)C(N(R_{6b}))(N(R_{6b})₂), -N(R_{6b})C(N(R_{6b}))(N(R_{6b})₂), =O, =S, =N(R₁), =N(R_{6b}) or W₅;

R₄ is independently alkyl of 1 to 12 carbon atoms, alkenyl of 2 to 12 carbon atoms, or alkynyl of 2 to 12 carbon atoms;

R₅ is independently R₄ wherein each R₄ is substituted with 0 to 3 R₃ groups;

R_{5a} is independently alkylene of 1 to 12 carbon atoms, alkenylene of 2 to 12 carbon atoms, or alkynylene of 2-12 carbon atoms any one of which alkylene, alkenylene or alkynylene is substituted with 0-3 R₃ groups;

R_{6a} is independently H or an ether- or ester-forming group;

R_{6b} is independently H, a protecting group for amino or the residue of a carboxyl-containing compound;

R_{6c} is independently H or the residue of an amino-containing compound;

W₃ is W₄ or W₅;

W₄ is R₅, -C(Y₁)R₅, -C(Y₁)W₅, -SO₂R₅, or -SO₂W₅;

W₅ is carbocycle or heterocycle wherein W₅ is independently substituted with 0 to 3 R₂ groups;

m₁ is independently an integer from 0 to 12, wherein the sum of all m₁'s within each individual embodiment of A₁, A₂ or A₃ is 12 or less; and

m₂ is independently an integer from 0 to 2.

In an embodiment

A₁ is -(C(R₂)(R₂))_{m1}-W₃, wherein W₃ is substituted with 1 A₃ group;

A₂ is -(C(R₂)(R₂))_{m1}-W₃; and

A₃ is -(C(R₂)(R₂))_{m1}-P(Y₁)(Y₁R_{6a})(Y₁R_{6a}).

Protecting Groups

The chemical substructure of a protecting group varies widely. One function of a protecting group is to serve as intermediates in the synthesis of the parental drug substance. Chemical protecting groups and strategies for protection/deprotection are well known in the art. See: "Protective Groups in Organic Chemistry", Theodora W. Greene (John Wiley & Sons, Inc., New York, 1991). Protecting groups are often utilized to mask the reactivity of certain functional groups, to assist in the efficiency of desired chemical reactions, e.g. making and breaking chemical bonds in an ordered and planned fashion. Protection of functional groups of a group, such as the polarity, lipophilicity (hydrophobicity), and other properties which can be measured by common analytical tools. Chemically protected intermediates may themselves be biologically active or inactive. Protected compounds may also exhibit altered, and in some cases, optimized properties *in vitro* and *in vivo*, such as passage through cellular membranes and resistance to enzymatic degradation or sequestration. In this role, protected compounds may in themselves exhibit therapeutic activity and need not be limited to the role of chemical intermediates or precursors. The protecting group need not be physiologically acceptable upon deprotection, although in general it is more desirable if such products are pharmacologically innocuous. A compound alters other physical properties besides the reactivity of the protected function.

In the context of the present invention, embodiments of protecting groups include prodrug moieties and chemical protecting groups.

Protecting groups are available, commonly known and used, and are optionally used to prevent side reactions with the protected group during synthetic procedures, i.e. routes or methods to prepare the compounds of the invention. For the most part the decision as to which groups to protect, when to do so, and the nature of the chemical protecting group "PRT" will be dependent upon the chemistry of the reaction to be protected against (e.g., acidic, basic, oxidative, reductive or other conditions) and the intended direction of the synthesis. The PRT groups do not need to be, and generally are not, the same if the compound is substituted with multiple PRT. In general, PRT will be used to protect

functional groups such as carboxyl, hydroxyl or amino groups and to thus prevent side reactions or to otherwise facilitate the synthetic efficiency. The order of deprotection to yield free, deprotected groups is dependent upon the intended direction of the synthesis and the reaction conditions to be encountered, and may occur in any order as determined by the artisan.

Various functional groups of the compounds of the invention may be protection. For example, protecting groups for -OH groups (whether hydroxyl, carboxylic acid, phosphonic acid, or other functions) are embodiments of "ether- or ester-forming groups". Ether- or ester-forming groups are capable of functioning as chemical protecting groups in the synthetic schemes set forth herein. However, some hydroxyl and thio protecting groups are neither ether- nor ester-forming groups, as will be understood by those skilled in the art, and are included with amides, discussed below.

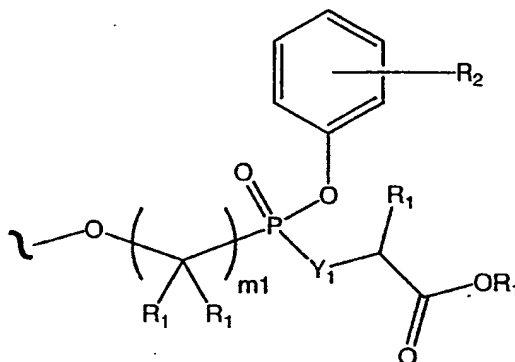
A very large number of hydroxyl protecting groups and amide-forming groups and corresponding chemical cleavage reactions are described in "Protective Groups in Organic Chemistry", Theodora W. Greene (John Wiley & Sons, Inc., New York, 1991, ISBN 0-471-62301-6) ("Greene"). See also Kocienski, Philip J.; "Protecting Groups" (Georg Thieme Verlag Stuttgart, New York, 1994), which is incorporated by reference in its entirety herein. In particular Chapter 1, Protecting Groups: An Overview, pages 1-20, Chapter 2, Hydroxyl Protecting Groups, pages 21-94, Chapter 3, Diol Protecting Groups, pages 95-117, Chapter 4, Carboxyl Protecting Groups, pages 118-154, Chapter 5, Carbonyl Protecting Groups, pages 155-184. For protecting groups for carboxylic acid, phosphonic acid, phosphonate, sulfonic acid and other protecting groups for acids see Greene as set forth below. Such groups include by way of example and not limitation, esters, amides, hydrazides, and the like.

Ether- and Ester-forming protecting groups

Ester-forming groups include: (1) phosphonate ester-forming groups, such as phosphoramidate esters, phosphorothioate esters, phosphonate esters, and phosphon-bis-amidates; (2) carboxyl ester-forming groups, and (3) sulphur ester-forming groups, such as sulphonate, sulfate, and sulfinates.

The phosphonate moieties of the compounds of the invention may or may not be prodrug moieties, i.e. they may or may be susceptible to hydrolytic or enzymatic cleavage or modification. Certain phosphonate moieties are stable under most or nearly all metabolic conditions. For example, a dialkylphosphonate, where the alkyl groups are two or more carbons, may have appreciable stability *in vivo* due to a slow rate of hydrolysis.

Within the context of phosphonate prodrug moieties, a large number of structurally-diverse prodrugs have been described for phosphonic acids (Freeman and Ross in Progress in Medicinal Chemistry 34: 112-147 (1997) and are included within the scope of the present invention. An exemplary embodiment of a phosphonate ester-forming group is the phenyl carbocycle in substructure A₃ having the formula:



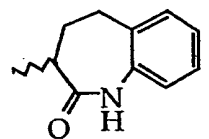
wherein m_1 is 1, 2, 3, 4, 5, 6, 7 or 8, and the phenyl carbocycle is substituted with 0 to 3 R_2 groups. Also, in this embodiment, where Y_1 is O, a lactate ester is formed. Alternatively, where Y_1 is $N(R_2)$, $N(OR_2)$ or $N(N(R_2)_2)$, then phosphoramidate esters result. R_1 may be H or C_1 - C_{12} alkyl.

In its ester-forming role, a protecting group typically is bound to any acidic group such as, by way of example and not limitation, a $-CO_2H$ or $-C(S)OH$ group, thereby resulting in $-CO_2R^x$ where R^x is defined herein. Also, R^x for example includes the enumerated ester groups of WO 95/07920.

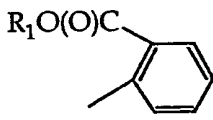
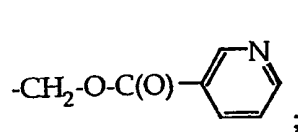
Examples of protecting groups include:

C_3 - C_{12} heterocycle (described above) or aryl. These aromatic groups optionally are polycyclic or monocyclic. Examples include phenyl, spiryl, 2- and 3-pyrrolyl, 2- and 3-thienyl, 2- and 4-imidazolyl, 2-, 4- and 5-oxazolyl, 3- and 4-isoxazolyl, 2-, 4- and 5-thiazolyl, 3-, 4-

and 5-isothiazolyl, 3- and 4-pyrazolyl, 1-, 2-, 3- and 4-pyridinyl, and 1-, 2-, 4- and 5-pyrimidinyl, C₃-C₁₂ heterocycle or aryl substituted with halo, R¹, R¹-O-C₁-C₁₂ alkylene, C₁-C₁₂ alkoxy, CN, NO₂, OH, carboxy, carboxyester, thiol, thioester, C₁-C₁₂ haloalkyl (1-6 halogen atoms), C₂-C₁₂ alkenyl or C₂-C₁₂ alkynyl. Such groups include 2-, 3- and 4-alkoxyphenyl (C₁-C₁₂ alkyl), 2-, 3- and 4-methoxyphenyl, 2-, 3- and 4-ethoxyphenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-diethoxyphenyl, 2- and 3-carboethoxy-4-hydroxyphenyl, 2- and 3-ethoxy-4-hydroxyphenyl, 2- and 3-ethoxy-5-hydroxyphenyl, 2- and 3-ethoxy-6-hydroxyphenyl, 2-, 3- and 4-O-acetylphenyl, 2-, 3- and 4-dimethylaminophenyl, 2-, 3- and 4-methylmercaptophenyl, 2-, 3- and 4-halophenyl (including 2-, 3- and 4-fluorophenyl and 2-, 3- and 4-chlorophenyl), 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethylphenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-biscarboxyethylphenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dimethoxyphenyl, 2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-dihalophenyl (including 2,4-difluorophenyl and 3,5-difluorophenyl), 2-, 3- and 4-haloalkylphenyl (1 to 5 halogen atoms, C₁-C₁₂ alkyl including 4-trifluoromethylphenyl), 2-, 3- and 4-cyanophenyl, 2-, 3- and 4-nitrophenyl, 2-, 3- and 4-haloalkylbenzyl (1 to 5 halogen atoms, C₁-C₁₂ alkyl including 4-trifluoromethylbenzyl and 2-, 3- and 4-trichloromethylphenyl and 2-, 3- and 4-trichloromethylphenyl), 4-N-methylpiperidinyl, 3-N-methylpiperidinyl, 1-ethylpiperazinyl, benzyl, alkylsalicylphenyl (C₁-C₄ alkyl, including 2-, 3- and 4-ethylsalicylphenyl), 2-, 3- and 4-acetylphenyl, 1,8-dihydroxynaphthyl (-C₁₀H₆-OH) and aryloxy ethyl [C₆-C₉ aryl (including phenoxy ethyl)], 2,2'-dihydroxybiphenyl, 2-, 3- and 4-N,N-dialkylaminophenol, -C₆H₄CH₂-N(CH₃)₂,



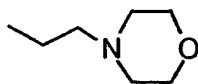
trimethoxybenzyl, triethoxybenzyl, 2-alkyl pyridinyl (C₁₋₄ alkyl);



; C₄ - C₈ esters of 2-carboxyphenyl; and C₁-

C₄ alkylene-C₃-C₆ aryl (including benzyl, -CH₂-pyrrolyl, -CH₂-thienyl, -CH₂-imidazolyl, -CH₂-oxazolyl, -CH₂-isoxazolyl, -CH₂-thiazolyl, -CH₂-isothiazolyl, -CH₂-pyrazolyl, -CH₂-pyridinyl and -CH₂-pyrimidinyl) substituted in the aryl moiety by 3 to 5 halogen atoms or 1 to 2 atoms or groups selected from halogen, C₁-C₁₂ alkoxy (including methoxy and ethoxy), cyano, nitro, OH, C₁-C₁₂ haloalkyl (1 to 6 halogen atoms; including -CH₂CCl₃), C₁-C₁₂ alkyl (including methyl and ethyl), C₂-C₁₂ alkenyl or C₂-C₁₂ alkynyl; alkoxy ethyl [C₁-C₆ alkyl including -CH₂-CH₂-O-CH₃ (methoxy ethyl)]; alkyl substituted by any of the groups set forth

above for aryl, in particular OH or by 1 to 3 halo atoms (including $-\text{CH}_3$, $-\text{CH}(\text{CH}_3)_2$, $-\text{C}(\text{CH}_3)_3$, $-\text{CH}_2\text{CH}_3$, $-(\text{CH}_2)_2\text{CH}_3$, $-(\text{CH}_2)_3\text{CH}_3$, $-(\text{CH}_2)_4\text{CH}_3$, $-(\text{CH}_2)_5\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{F}$, $-\text{CH}_2\text{CH}_2\text{Cl}$, $-\text{CH}_2\text{CF}_3$, and $-\text{CH}_2\text{CCl}_3$);



; -N-2-propylmorpholino, 2,3-dihydro-6-hydroxyindene, sesamol, catechol monoester, $-\text{CH}_2-\text{C}(\text{O})-\text{N}(\text{R}^1)_2$, $-\text{CH}_2-\text{S}(\text{O})(\text{R}^1)$, $-\text{CH}_2-\text{S}(\text{O})_2(\text{R}^1)$, $-\text{CH}_2-\text{CH}(\text{OC}(\text{O})\text{CH}_2\text{R}^1)-\text{CH}_2(\text{OC}(\text{O})\text{CH}_2\text{R}^1)$, cholesteryl, enolpyruvate ($\text{HOOC}-\text{C}(\text{=CH}_2)-$), glycerol;

a 5 or 6 carbon monosaccharide, disaccharide or oligosaccharide (3 to 9 monosaccharide residues);

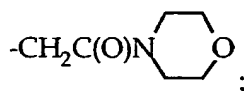
triglycerides such as α -D- β -diglycerides (wherein the fatty acids composing glyceride lipids generally are naturally occurring saturated or unsaturated C₆₋₂₆, C₆₋₁₈ or C₆₋₁₀ fatty acids such as linoleic, lauric, myristic, palmitic, stearic, oleic, palmitoleic, linolenic and the like fatty acids) linked to acyl of the parental compounds herein through a glyceryl oxygen of the triglyceride;

phospholipids linked to the carboxyl group through the phosphate of the phospholipid;

phthalidyl (shown in Fig. 1 of Clayton et al., *Antimicrob. Agents Chemo.* (1974)

5(6):670-671;

cyclic carbonates such as (5-R_d-2-oxo-1,3-dioxolen-4-yl) methyl esters (Sakamoto et al., *Chem. Pharm. Bull.* (1984) 32(6)2241-2248) where R_d is R₁, R₄ or aryl; and



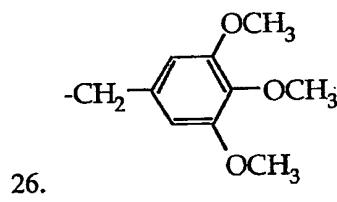
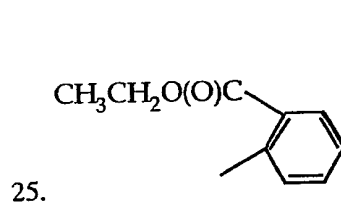
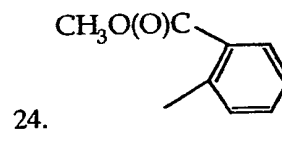
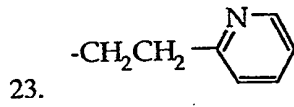
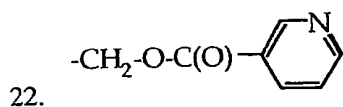
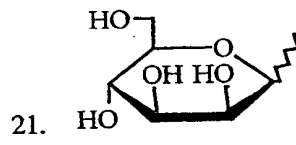
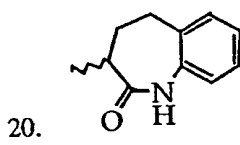
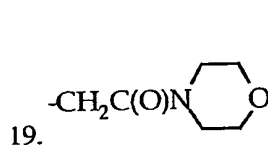
The hydroxyl groups of the compounds of this invention optionally are substituted with one of groups III, IV or V disclosed in WO 94/21604, or with isopropyl.

As further embodiments, Table A lists examples of protecting group ester moieties that for example can be bonded via oxygen to $-\text{C}(\text{O})\text{O}-$ and $-\text{P}(\text{O})(\text{O}-)_2$ groups. Several amidates also are shown, which are bound directly to $-\text{C}(\text{O})-$ or $-\text{P}(\text{O})_2$. Esters of structures 1-5, 8-10 and 16, 17, 19-22 are synthesized by reacting the compound herein having a free hydroxyl with the corresponding halide (chloride or acyl chloride and the like) and N,N-dicyclohexyl-N-morpholine carboxamidine (or another base such as DBU, triethylamine, CsCO_3 , N,N-dimethylaniline and the like) in DMF (or other solvent such as acetonitrile or N-methylpyrrolidone). When the compound to be protected is a phosphonate, the esters of structures 5-7, 11, 12, 21, and 23-26 are synthesized by reaction of the alcohol or alkoxide salt

(or the corresponding amines in the case of compounds such as 13, 14 and 15) with the monochlorophosphonate or dichlorophosphonate (or another activated phosphonate).

TABLE A

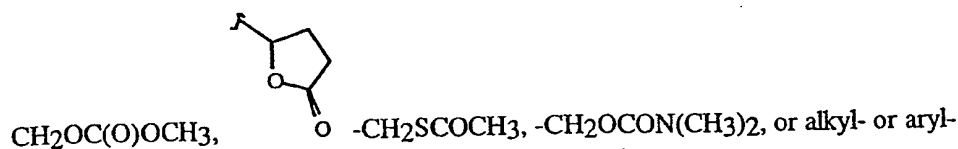
- | | |
|--|---|
| 1. $-\text{CH}_2-\text{C}(\text{O})-\text{N}(\text{R}_1)_2^*$ | 10. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{C}(\text{CH}_3)_3$ |
| 2. $-\text{CH}_2-\text{S}(\text{O})(\text{R}_1)$ | 11. $-\text{CH}_2-\text{CCl}_3$ |
| 3. $-\text{CH}_2-\text{S}(\text{O})_2(\text{R}_1)$ | 12. $-\text{C}_6\text{H}_5$ |
| 4. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{CH}_2-\text{C}_6\text{H}_5$ | 13. $-\text{NH}-\text{CH}_2-\text{C}(\text{O})\text{O}-\text{CH}_2\text{CH}_3$ |
| 5. 3-cholesteryl | 14. $-\text{N}(\text{CH}_3)-\text{CH}_2-\text{C}(\text{O})\text{O}-\text{CH}_2\text{CH}_3$ |
| 6. 3-pyridyl | 15. $-\text{NHR}_1$ |
| 7. N-ethylmorpholino | 16. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{C}_{10}\text{H}_{15}$ |
| 8. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{C}_6\text{H}_5$ | 17. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{CH}(\text{CH}_3)_2$ |
| 9. $-\text{CH}_2-\text{O}-\text{C}(\text{O})-\text{CH}_2\text{CH}_3$ | 18. $-\text{CH}_2-\text{C}\equiv\text{H}(\text{OC}(\text{O})\text{CH}_2\text{R}_1)-\text{CH}_2-$
$-(\text{OC}(\text{O})\text{CH}_2\text{R}_1)^*$ |



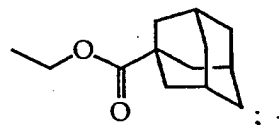
- chiral center is (R), (S) or racemate.

Other esters that are suitable for use herein are described in EP 632048.

Protecting groups also includes "double ester" forming profunctionalities such as -



acyloxyalkyl groups of the structure $-\text{CH}(\text{R}^1 \text{ or } \text{W}^5)\text{O}((\text{CO})\text{R}^{37})$ or $-\text{CH}(\text{R}^1 \text{ or } \text{W}^5)((\text{CO})\text{OR}^{38})$ (linked to oxygen of the acidic group) wherein R^{37} and R^{38} are alkyl, aryl, or alkylaryl groups (see U.S. patent 4,968,788). Frequently R^{37} and R^{38} are bulky groups such as branched alkyl, ortho-substituted aryl, meta-substituted aryl, or combinations thereof, including normal, secondary, iso- and tertiary alkyls of 1-6 carbon atoms. An example is the pivaloyloxymethyl group. These are of particular use with prodrugs for oral administration. Examples of such useful protecting groups are alkylacyloxymethyl esters and



their derivatives, including $-\text{CH}(\text{CH}_2\text{CH}_2\text{OCH}_3)\text{OC}(\text{O})\text{C}(\text{CH}_3)_3$, $-\text{CH}_2\text{OC}(\text{O})\text{C}_{10}\text{H}_{15}$, $-\text{CH}_2\text{OC}(\text{O})\text{C}(\text{CH}_3)_3$, $-\text{CH}(\text{CH}_2\text{OCH}_3)\text{OC}(\text{O})\text{C}(\text{CH}_3)_3$, $-\text{CH}(\text{CH}(\text{CH}_3)_2)\text{OC}(\text{O})\text{C}(\text{CH}_3)_3$, $-\text{CH}_2\text{OC}(\text{O})\text{CH}_2\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{OC}(\text{O})\text{C}_6\text{H}_{11}$, $-\text{CH}_2\text{OC}(\text{O})\text{C}_6\text{H}_5$, $-\text{CH}_2\text{OC}(\text{O})\text{C}_{10}\text{H}_{15}$, $-\text{CH}_2\text{OC}(\text{O})\text{CH}_2\text{CH}_3$, $-\text{CH}_2\text{OC}(\text{O})\text{CH}(\text{CH}_3)_2$, $-\text{CH}_2\text{OC}(\text{O})\text{C}(\text{CH}_3)_3$ and $-\text{CH}_2\text{OC}(\text{O})\text{CH}_2\text{C}_6\text{H}_5$.

For prodrug purposes, the ester typically chosen is one heretofore used for antibiotic drugs, in particular the cyclic carbonates, double esters, or the phthalidyl, aryl or alkyl esters.

In some embodiments the protected acidic group is an ester of the acidic group and is the residue of a hydroxyl-containing functionality. In other embodiments, an amino compound is used to protect the acid functionality. The residues of suitable hydroxyl or amino-containing functionalities are set forth above or are found in WO 95/07920. Of particular interest are the residues of amino acids, amino acid esters, polypeptides, or aryl alcohols. Typical amino acid, polypeptide and carboxyl-esterified amino acid residues are described on pages 11-18 and related text of WO 95/07920 as groups L1 or L2. WO 95/07920 expressly teaches the amidates of phosphonic acids, but it will be understood that such amidates are formed with any of the acid groups set forth herein and the amino acid residues set forth in WO 95/07920.

Typical esters for protecting acidic functionalities are also described in WO 95/07920, again understanding that the same esters can be formed with the acidic groups herein as with the phosphonate of the '920 publication. Typical ester groups are defined at least on WO 95/07920 pages 89-93 (under R^{31} or R^{35}), the table on page 105, and pages 21-23 (as R). Of particular interest are esters of unsubstituted aryl such as phenyl or arylalkyl such benzyl, or hydroxy-, halo-, alkoxy-, carboxy- and/or alkylestercarboxy-substituted aryl or alkylaryl,

especially phenyl, ortho-ethoxyphenyl, or C₁-C₄ alkylestercarboxyphenyl (salicylate C₁-C₁₂ alkylesters).

The protected acidic groups, particularly when using the esters or amides of WO 95/07920, are useful as prodrugs for oral administration. However, it is not essential that the acidic group be protected in order for the compounds of this invention to be effectively administered by the oral route. When the compounds of the invention having protected groups, in particular amino acid amidates or substituted and unsubstituted aryl esters are administered systemically or orally they are capable of hydrolytic cleavage *in vivo* to yield the free acid.

One or more of the acidic hydroxyls are protected. If more than one acidic hydroxyl is protected then the same or a different protecting group is employed, e.g., the esters may be different or the same, or a mixed amidate and ester may be used.

Typical hydroxy protecting groups described in Greene (pages 14-118) include substituted methyl and alkyl ethers, substituted benzyl ethers, silyl ethers, esters including sulfonic acid esters, and carbonates. For example:

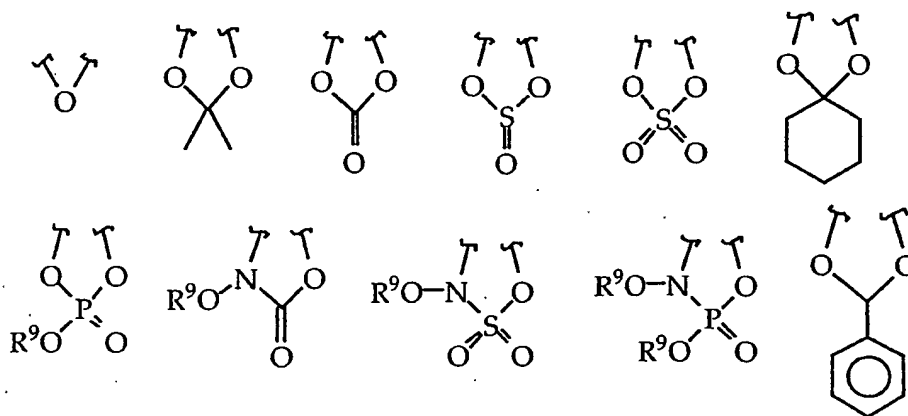
- Ethers (methyl, *t*-butyl, allyl);
- Substituted Methyl Ethers (Methoxymethyl, Methylthiomethyl, *t*-Butylthiomethyl, (Phenyldimethylsilyl)methoxymethyl, Benzyloxymethyl, *p*-Methoxybenzyloxymethyl, (4-Methoxyphenoxy)methyl, Guaiacolmethyl, *t*-Butoxymethyl, 4-Pentenylloxymethyl, Siloxymethyl, 2-Methoxyethoxymethyl, 2,2,2-Trichloroethoxymethyl, Bis(2-chloroethoxy)methyl, 2-(Trimethylsilyl)ethoxymethyl, Tetrahydropyranyl, 3-Bromotetrahydropyranyl, Tetrahydrothiopyranyl, 1-Methoxycyclohexyl, 4-Methoxytetrahydropyranyl, 4-Methoxytetrahydrothiopyranyl, 4-Methoxytetrahydrothiopyranyl *S,S*-Dioxido, 1-[(2-Chloro-4-methyl)phenyl]-4-methoxypiperidin-4-yl, 1,4-Dioxan-2-yl, Tetrahydrofuranlyl, Tetrahydrothiofuranlyl, 2,3,3a,4,5,6,7,7a-Octahydro-7,8,8-trimethyl-4,7-methanobenzofuran-2-yl));
- Substituted Ethyl Ethers (1-Ethoxyethyl, 1-(2-Chloroethoxy)ethyl, 1-Methyl-1-methoxyethyl, 1-Methyl-1-benzyloxyethyl, 1-Methyl-1-benzyloxy-2-fluoroethyl, 2,2,2-Trichloroethyl, 2-Trimethylsilylethyl, 2-(Phenylselenyl)ethyl,
- *p*-Chlorophenyl, *p*-Methoxyphenyl, 2,4-Dinitrophenyl, Benzyl);
- Substituted Benzyl Ethers (*p*-Methoxybenzyl, 3,4-Dimethoxybenzyl, *o*-Nitrobenzyl, *p*-

- Nitrobenzyl, *p*-Halobenzyl, 2,6-Dichlorobenzyl, *p*-Cyanobenzyl, *p*-Phenylbenzyl, 2- and 4-Picolyl, 3-Methyl-2-picolyl *N*-Oxido, Diphenylmethyl, *p,p'*-Dinitrobenzhydrl, 5-Dibenzosuberyl, Triphenylmethyl, α -Naphthyldiphenylmethyl, *p*-methoxyphenyldiphenylmethyl, Di(*p*-methoxyphenyl)phenylmethyl, Tri(*p*-methoxyphenyl)methyl, 4-(4'-Bromophenacyloxy)phenyldiphenylmethyl, 4,4',4''-Tris(4,5-dichlorophthalimidophenyl)methyl, 4,4',4''-Tris(levulinoyloxyphenyl)methyl, 4,4',4''-Tris(benzoyloxyphenyl)methyl, 3-(Imidazol-1-ylmethyl)bis(4',4''-dimethoxyphenyl)methyl, 1,1-Bis(4-methoxyphenyl)-1'-pyrenylmethyl, 9-Anthryl, 9-(9-Phenyl)xanthenyl, 9-(9-Phenyl-10-oxo)anthryl, 1,3-Benzodithiolan-2-yl, Benzisothiazolyl *S,S*-Dioxido);
- Silyl Ethers (Trimethylsilyl, Triethylsilyl, Triisopropylsilyl, Dimethylisopropylsilyl, Diethylisopropylsilyl, Dimethylhexylsilyl, *t*-Butyldimethylsilyl, *t*-Butyldiphenylsilyl, Tribenzylsilyl, Tri-*p*-xylylsilyl, Triphenylsilyl, Diphenylmethylsilyl, *t*-Butylmethoxyphenylsilyl);
 - Esters (Formate, Benzoylformate, Acetate, Choroacetate, Dichloroacetate, Trichloroacetate, Trifluoroacetate, Methoxyacetate, Triphenylmethoxyacetate, Phenoxyacetate, *p*-Chlorophenoxyacetate, *p*-poly-Phenylacetate, 3-Phenylpropionate, 4-Oxopentanoate (Levulinate), 4,4-(Ethylenedithio)pentanoate, Pivaloate, Adamantoate, Crotonate, 4-Methoxycrotonate, Benzoate, *p*-Phenylbenzoate, 2,4,6-Trimethylbenzoate (Mesitoate));
 - Carbonates (Methyl, 9-Fluorenylmethyl, Ethyl, 2,2,2-Trichloroethyl, 2-(Trimethylsilyl)ethyl, 2-(Phenylsulfonyl)ethyl, 2-(Triphenylphosphonio)ethyl, Isobutyl, Vinyl, Allyl, *p*-Nitrophenyl, Benzyl, *p*-Methoxybenzyl, 3,4-Dimethoxybenzyl, *o*-Nitrobenzyl, *p*-Nitrobenzyl, *S*-Benzyl Thiocarbonate, 4-Ethoxy-1-naphthyl, Methyl Dithiocarbonate);
 - Groups With Assisted Cleavage (2-Iodobenzoate, 4-Azidobutyrate, 4-Nitro-4-methylpentanoate, *o*-(Dibromomethyl)benzoate, 2-Formylbenzenesulfonate, 2-(Methylthiomethoxy)ethyl Carbonate, 4-(Methylthiomethoxy)butyrate, 2-(Methylthiomethoxymethyl)benzoate); Miscellaneous Esters (2,6-Dichloro-4-methylphenoxyacetate, 2,6-Dichloro-4-(1,1,3,3 tetramethylbutyl)phenoxyacetate, 2,4-Bis(1,1-dimethylpropyl)phenoxyacetate, Chlorodiphenylacetate, Isobutyrate, Monosuccinate, (*E*)-2-Methyl-2-butenate (Tigloate), *o*-(Methoxycarbonyl)benzoate, *p*-poly-Benzoate, α -Naphthoate, Nitrate, Alkyl *N,N,N',N'*-Tetramethylphosphorodiamidate, *N*-Phenylcarbamate, Borate, Dimethylphosphinothioyl, 2,4-Dinitrophenylsulfenate); and
 - Sulfonates (Sulfate, Methanesulfonate (Mesylate), Benzylsulfonate, Tosylate).

- Typical 1,2-diol protecting groups (thus, generally where two OH groups are taken together with the protecting functionality) are described in Greene at pages 118-142 and include Cyclic Acetals and Ketals (Methylene, Ethylidene, 1-*t*-Butylethylidene, 1-Phenylethylidene, (4-Methoxyphenyl)ethylidene, 2,2,2-Trichloroethylidene, Acetonide (Isopropylidene), Cyclopentylidene, Cyclohexylidene, Cycloheptylidene, Benzylidene, *p*-Methoxybenzylidene, 2,4-Dimethoxybenzylidene, 3,4-Dimethoxybenzylidene, 2-Nitrobenzylidene); Cyclic Ortho Esters (Methoxymethylene, Ethoxymethylene, Dimethoxymethylene, 1-Methoxyethylidene, 1-Ethoxyethylidene, 1,2-Dimethoxyethylidene, α -Methoxybenzylidene, 1-(*N,N*-Dimethylamino)ethylidene Derivative, α -(*N,N*-Dimethylamino)benzylidene Derivative, 2-Oxacyclopentylidene); Silyl Derivatives (Di-*t*-butylsilylene Group, 1,3-(1,1,3,3-Tetraisopropylidisiloxanylidene), and Tetra-*t*-butoxydisiloxane-1,3-diylidene), Cyclic Carbonates, Cyclic Boronates, Ethyl Boronate and Phenyl Boronate.

More typically, 1,2-diol protecting groups include those shown in Table B, still more typically, epoxides, acetonides, cyclic ketals and aryl acetals.

Table B



wherein R⁹ is C₁-C₆ alkyl.

Amino protecting groups

Another set of protecting groups include any of the typical amino protecting groups described by Greene at pages 315-385. They include:

- Carbamates: (methyl and ethyl, 9-fluorenylmethyl, 9(2-sulfo)fluorenylmethyl, 9-(2,7-dibromo)fluorenylmethyl, 2,7-di-*t*-butyl-[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl, 4-methoxyphenacyl);
- Substituted Ethyl: (2,2,2-trichloroethyl, 2-trimethylsilylethyl, 2-phenylethyl, 1-(1-adamantyl)-1-methylethyl, 1,1-dimethyl-2-haloethyl, 1,1-dimethyl-2,2-dibromoethyl, 1,1-dimethyl-2,2,2-trichloroethyl, 1-methyl-1-(4-biphenyl)ethyl, 1-(3,5-di-*t*-butylphenyl)-1-methylethyl, 2-(2'- and 4'-pyridyl)ethyl, 2-(*N,N*-dicyclohexylcarboxamido)ethyl, *t*-butyl, 1-adamantyl, vinyl, allyl, 1-isopropylallyl, cinnamyl, 4-nitrocinnamyl, 8-quinolyl, *N*-hydroxypiperidiny, alkylidithio, benzyl, *p*-methoxybenzyl, *p*-nitrobenzyl, *p*-bromobenzyl, *p*-chlorobenzyl, 2,4-dichlorobenzyl, 4-methylsulfinylbenzyl, 9-anthrylmethyl, diphenylmethyl);
- Groups With Assisted Cleavage: (2-methylthioethyl, 2-methylsulfonylethyl, 2-(*p*-toluenesulfonyl)ethyl, [2-(1,3-dithianyl)]methyl, 4-methylthiophenyl, 2,4-dimethylthiophenyl, 2-phosphonioethyl, 2-triphenylphosphonioisopropyl, 1,1-dimethyl-2-cyanoethyl, *m*-chloro-*p*-acyloxybenzyl, *p*-(dihydroxyboryl)benzyl, 5-benzisoxazolymethyl, 2-(trifluoromethyl)-6-chromonylmethyl);
- Groups Capable of Photolytic Cleavage: (*m*-nitrophenyl, 3,5-dimethoxybenzyl, *o*-nitrobenzyl, 3,4-dimethoxy-6-nitrobenzyl, phenyl(*o*-nitrophenyl)methyl); Urea-Type Derivatives (phenothiazinyl-(10)-carbonyl, *N'*-*p*-toluenesulfonylaminocarbonyl, *N'*-

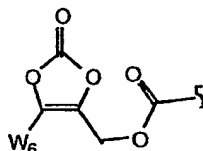
phenylaminothiocarbonyl);

- Miscellaneous Carbamates: (*t*-amyl, *S*-benzyl thiocarbamate, *p*-cyanobenzyl, cyclobutyl, cyclohexyl, cyclopentyl, cyclopropylmethyl, *p*-decyloxybenzyl, diisopropylmethyl, 2,2-dimethoxycarbonylvinyl, *o*-(*N,N*-dimethylcarboxamido)benzyl, 1,1-dimethyl-3-(*N,N*-dimethylcarboxamido)propyl, 1,1-dimethylpropynyl, di(2-pyridyl)methyl, 2-furanylmethyl, 2-Iodoethyl, Isobornyl, Isobutyl, Isonicotinyl, *p*-(*p'*-Methoxyphenylazo)benzyl, 1-methylcyclobutyl, 1-methylcyclohexyl, 1-methyl-1-cyclopropylmethyl, 1-methyl-1-(3,5-dimethoxyphenyl)ethyl, 1-methyl-1-(*p*-phenylazophenyl)ethyl, 1-methyl-1-phenylethyl, 1-methyl-1-(4-pyridyl)ethyl, phenyl, *p*-(phenylazo)benzyl, 2,4,6-tri-*t*-butylphenyl, 4-(trimethylammonium)benzyl, 2,4,6-trimethylbenzyl);
- Amides: (*N*-formyl, *N*-acetyl, *N*-choroacetyl, *N*-trichloroacetyl, *N*-trifluoroacetyl, *N*-phenylacetyl, *N*-3-phenylpropionyl, *N*-picolinoyl, *N*-3-pyridylcarboxamide, *N*-benzoylphenylalanyl, *N*-benzoyl, *N*-*p*-phenylbenzoyl);
- Amides With Assisted Cleavage: (*N*-*o*-nitrophenylacetyl, *N*-*o*-nitrophenoxyacetyl, *N*-acetoacetyl, (*N'*-dithiobenzoyloxycarbonylamino)acetyl, *N*-3-(*p*-hydroxyphenyl)propionyl, *N*-3-(*o*-nitrophenyl)propionyl, *N*-2-methyl-2-(*o*-nitrophenoxy)propionyl, *N*-2-methyl-2-(*o*-phenylazophenoxy)propionyl, *N*-4-chlorobutyryl, *N*-3-methyl-3-nitrobutyryl, *N*-*o*-nitrocinnamoyl, *N*-acetylmethionine, *N*-*o*-nitrobenzoyl, *N*-*o*-(benzoyloxymethyl)benzoyl, 4,5-diphenyl-3-oxazolin-2-one);
- Cyclic Imide Derivatives: (*N*-phthalimide, *N*-dithiasuccinoyl, *N*-2,3-diphenylmaleoyl, *N*-2,5-dimethylpyrrolyl, *N*-1,1,4,4-tetramethyldisilylazacyclopentane adduct, 5-substituted 1,3-dimethyl-1,3,5-triazacyclohexan-2-one, 5-substituted 1,3-dibenzyl-1,3,5-triazacyclohexan-2-one, 1-substituted 3,5-dinitro-4-pyridonyl);
- *N*-Alkyl and *N*-Aryl Amines: (*N*-methyl, *N*-allyl, *N*-[2-(trimethylsilyl)ethoxy]methyl, *N*-3-acetoxypentyl, *N*-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl), Quaternary Ammonium Salts, *N*-benzyl, *N*-di(4-methoxyphenyl)methyl, *N*-5-dibenzosuberyl, *N*-triphenylmethyl, *N*-(4-methoxyphenyl)diphenylmethyl, *N*-9-phenylfluorenyl, *N*-2,7-dichloro-9-fluorenylmethylene, *N*-ferrocenylmethyl, *N*-2-picolyamine *N'*-oxide);
- Imine Derivatives: (*N*-1,1-dimethylthiomethylene, *N*-benzylidene, *N*-*p*-methoxybenzylidene, *N*-diphenylmethylene, *N*-[(2-pyridyl)mesityl]methylene, *N*-(*N,N'*-dimethylaminomethylene, *N,N'*-isopropylidene, *N*-*p*-nitrobenzylidene, *N*-salicylidene, *N*-5-chlorosalicylidene, *N*-(5-chloro-2-hydroxyphenyl)phenylmethylene, *N*-cyclohexylidene);
- Enamine Derivatives: (*N*-(5,5-dimethyl-3-oxo-1-cyclohexenyl));
- *N*-Metal Derivatives (*N*-borane derivatives, *N*-diphenylborinic acid derivatives, *N*-

[phenyl(pentacarbonylchromium- or -tungsten)]carbenyl, *N*-copper or *N*-zinc chelate);

- N-N Derivatives: (*N*-nitro, *N*-nitroso, *N*-oxide);
- N-P Derivatives: (*N*-diphenylphosphinyl, *N*-dimethylthiophosphinyl, *N*-diphenylthiophosphinyl, *N*-dialkyl phosphoryl, *N*-dibenzyl phosphoryl, *N*-diphenyl phosphoryl);
- N-Si Derivatives, N-S Derivatives, and N-Sulfenyl Derivatives: (*N*-benzenesulfenyl, *N*-*o*-nitrobenzenesulfenyl, *N*-2,4-dinitrobenzenesulfenyl, *N*-pentachlorobenzenesulfenyl, *N*-2-nitro-4-methoxybenzenesulfenyl, *N*-triphenylmethylsulfenyl, *N*-3-nitropyridinesulfenyl); and *N*-sulfonyl Derivatives (*N*-*p*-toluenesulfonyl, *N*-benzenesulfonyl, *N*-2,3,6-trimethyl-4-methoxybenzenesulfonyl, *N*-2,4,6-trimethoxybenzenesulfonyl, *N*-2,6-dimethyl-4-methoxybenzenesulfonyl, *N*-pentamethylbenzenesulfonyl, *N*-2,3,5,6-tetramethyl-4-methoxybenzenesulfonyl, *N*-4-methoxybenzenesulfonyl, *N*-2,4,6-trimethylbenzenesulfonyl, *N*-2,6-dimethoxy-4-methylbenzenesulfonyl, *N*-2,2,5,7,8-pentamethylchroman-6-sulfonyl, *N*-methanesulfonyl, *N*- β -trimethylsilyethanesulfonyl, *N*-9-anthracenesulfonyl, *N*-4-(4',8'-dimethoxynaphthylmethyl)benzenesulfonyl, *N*-benzylsulfonyl, *N*-trifluoromethylsulfonyl, *N*-phenacylsulfonyl).

More typically, protected amino groups include carbamates and amides, still more typically, -NHC(O)R^1 or $\text{-N=CR}^1\text{N(R}^1\text{)}_2$. Another protecting group, also useful as a prodrug for amino or $\text{-NH(R}^5\text{)}$, is:



See for example Alexander, J. et al (1996) *J. Med. Chem.* 39:480-486.

Amino acid and polypeptide protecting group and conjugates

An amino acid or polypeptide protecting group of a compound of the invention has the structure $\text{R}^{15}\text{NHCH(R}^{16}\text{)C(O)-}$, where R^{15} is H, an amino acid or polypeptide residue, or R^5 , and R^{16} is defined below.

R^{16} is lower alkyl or lower alkyl ($\text{C}_1\text{-C}_6$) substituted with amino, carboxyl, amide, carboxyl ester, hydroxyl, $\text{C}_6\text{-C}_7$ aryl, guanidiny, imidazolyl, indolyl, sulfhydryl, sulfoxide, and/or alkylphosphate. R^{10} also is taken together with the amino acid α N to form a proline residue ($\text{R}^{10} = \text{-CH}_2\text{)}_3\text{-}$). However, R^{10} is generally the side group of a naturally-occurring amino acid such as H, -CH_3 , $\text{-CH(CH}_3\text{)}_2$, $\text{-CH}_2\text{-CH(CH}_3\text{)}_2$, $\text{-CHCH}_3\text{-CH}_2\text{-CH}_3$, $\text{-CH}_2\text{-C}_6\text{H}_5$,

-CH₂CH₂-S-CH₃, -CH₂OH, -CH(OH)-CH₃, -CH₂-SH, -CH₂-C₆H₄OH, -CH₂-CO-NH₂, -CH₂-CH₂-CO-NH₂, -CH₂-COOH, -CH₂-CH₂-COOH, -(CH₂)₄-NH₂ and -(CH₂)₃-NH-C(NH₂)-NH₂. R₁₀ also includes 1-guanidinoprop-3-yl, benzyl, 4-hydroxybenzyl, imidazol-4-yl, indol-3-yl, methoxyphenyl and ethoxyphenyl.

Another set of protecting groups include the residue of an amino-containing compound, in particular an amino acid, a polypeptide, a protecting group; -NHSO₂R, NHC(O)R, -N(R)₂, NH₂ or -NH(R)(H), whereby for example a carboxylic acid is reacted, i.e. coupled, with the amine to form an amide, as in C(O)NR₂. A phosphonic acid may be reacted with the amine to form a phosphoramidate, as in -P(O)(OR)(NR₂).

In general, amino acids have the structure R¹⁷C(O)CH(R¹⁶)NH-, where R¹⁷ is -OH, -OR, an amino acid or a polypeptide residue. Amino acids are low molecular weight compounds, on the order of less than about 1000 MW and which contain at least one amino or imino group and at least one carboxyl group. Generally the amino acids will be found in nature, i.e., can be detected in biological material such as bacteria or other microbes, plants, animals or man. Suitable amino acids typically are alpha amino acids, i.e. compounds characterized by one amino or imino nitrogen atom separated from the carbon atom of one carboxyl group by a single substituted or unsubstituted alpha carbon atom. Of particular interest are hydrophobic residues such as mono- or di-alkyl or aryl amino acids, cycloalkylamino acids and the like. These residues contribute to cell permeability by increasing the partition coefficient of the parental drug. Typically, the residue does not contain a sulfhydryl or guanidino substituent.

Naturally-occurring amino acid residues are those residues found naturally in plants, animals or microbes, especially proteins thereof. Polypeptides most typically will be substantially composed of such naturally-occurring amino acid residues. These amino acids are glycine, alanine, valine, leucine, isoleucine, serine, threonine, cysteine, methionine, glutamic acid, aspartic acid, lysine, hydroxylysine, arginine, histidine, phenylalanine, tyrosine, tryptophan, proline, asparagine, glutamine and hydroxyproline. Additionally, unnatural amino acids, for example, valanine, phenylglycine and homoarginine are also included. Commonly encountered amino acids that are not gene-encoded may also be used in the present invention. All of the amino acids used in the present invention may be either the D- or L- optical isomer. In addition, other peptidomimetics are also useful in the present invention. For a general

review, see Spatola, A. F., in *Chemistry and Biochemistry of Amino Acids, Peptides and Proteins*, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983).

When protecting groups are single amino acid residues or polypeptides they optionally are substituted at R^3 of substituents A^1 , A^2 or A^3 , or substituted at R_3 of substituents A_1 , A_2 or A_3 . These conjugates are produced by forming an amide bond between a carboxyl group of the amino acid (or C-terminal amino acid of a polypeptide for example). Similarly, conjugates are formed between R^3 or R_3 and an amino group of an amino acid or polypeptide. Generally, only one of any site in the parental molecule is amidated with an amino acid as described herein, although it is within the scope of this invention to introduce amino acids at more than one permitted site. Usually, a carboxyl group of R^3 is amidated with an amino acid. In general, the α -amino or α -carboxyl group of the amino acid or the terminal amino or carboxyl group of a polypeptide are bonded to the parental functionalities, i.e., carboxyl or amino groups in the amino acid side chains generally are not used to form the amide bonds with the parental compound (although these groups may need to be protected during synthesis of the conjugates as described further below).

With respect to the carboxyl-containing side chains of amino acids or polypeptides it will be understood that the carboxyl group optionally will be blocked, e.g. by R^1 , esterified with R^5 or amidated. Similarly, the amino side chains R^{16} optionally will be blocked with R^1 or substituted with R^5 .

Such ester or amide bonds with side chain amino or carboxyl groups, like the esters or amides with the parental molecule, optionally are hydrolyzable *in vivo* or *in vitro* under acidic (pH <3) or basic (pH >10) conditions. Alternatively, they are substantially stable in the gastrointestinal tract of humans but are hydrolyzed enzymatically in blood or in intracellular environments. The esters or amino acid or polypeptide amidates also are useful as intermediates for the preparation of the parental molecule containing free amino or carboxyl groups. The free acid or base of the parental compound, for example, is readily formed from the esters or amino acid or polypeptide conjugates of this invention by conventional hydrolysis procedures.

When an amino acid residue contains one or more chiral centers, any of the D, L, meso, threo or erythro (as appropriate) racemates, scalemates or mixtures thereof may be used. In general, if the intermediates are to be hydrolyzed non-enzymatically (as would be the

case where the amides are used as chemical intermediates for the free acids or free amines), D isomers are useful. On the other hand, the linker isomers are more versatile since they can be susceptible to both non-enzymatic and enzymatic hydrolysis, and are more efficiently transported by amino acid or dipeptidyl transport systems in the gastrointestinal tract.

Examples of suitable amino acids whose residues are represented by R^x or R^y include the following:

Glycine;

Aminopolycarboxylic acids, e.g., aspartic acid, β -hydroxyaspartic acid, glutamic acid, β -hydroxyglutamic acid, β -methylaspartic acid, β -methylglutamic acid, β , β -dimethylaspartic acid, γ -hydroxyglutamic acid, β , γ -dihydroxyglutamic acid, β -phenylglutamic acid, γ -methyleneglutamic acid, 3-aminoadipic acid, 2-aminopimelic acid, 2-aminosuberic acid and 2-aminosebacic acid;

Amino acid amides such as glutamine and asparagine;

Polyamino- or polybasic-monocarboxylic acids such as arginine, lysine, β -aminoalanine, γ -aminobutyric acid, ornithine, citrulline, homoarginine, homocitrulline, hydroxylysine, alcohxylysine and diaminobutyric acid;

Other basic amino acid residues such as histidine;

Diaminodicarboxylic acids such as α , α' -diaminosuccinic acid, α , α' -diaminoglutaric acid, α , α' -diaminoadipic acid, α , α' -diaminopimelic acid, α , α' -diamino- β -hydroxypimelic acid, α , α' -diaminosuberic acid, α , α' -diaminoazelaic acid, and α , α' -diaminosebacic acid;

Imino acids such as proline, hydroxyproline, alcohxyproline, γ -methylproline, pipercolic acid, 5-hydroxypipercolic acid, and azetidine-2-carboxylic acid;

A mono- or di-alkyl (typically C_1 - C_8 branched or normal) amino acid such as alanine, valine, leucine, allylglycine, butyric acid, norvaline, norleucine, heptyline, α -methylserine, α -amino- α -methyl- γ -hydroxyvaleric acid, α -amino- α -methyl- δ -hydroxyvaleric acid, α -amino- α -methyl- ϵ -hydroxycaproic acid, isovaline, α -methylglutamic acid, α -aminoisobutyric acid, α -aminodiethylacetic acid, α -aminodiisopropylacetic acid, α -aminodi-n-propylacetic acid, α -aminodiisobutylacetic acid, α -aminodi-n-butylacetic acid, α -aminoethylisopropylacetic acid, α -amino-n-propylacetic acid, α -aminodiisobutyric acid, α -methylaspartic acid, α -methylglutamic acid, 1-aminocyclopropane-1-carboxylic acid, isoleucine, alloisoleucine, *tert*-leucine, β -methyltryptophan and α -amino- β -ethyl- β -phenylpropionic acid;

β -phenylserine;

Aliphatic α -amino- β -hydroxy acids such as serine, β -hydroxyisoleucine, β -

hydroxynorleucine, β -hydroxynorvaline, and α -amino- β -hydroxystearic acid;

α -Amino, α -, γ -, δ - or ϵ -hydroxy acids such as homoserine, δ -hydroxynorvaline, γ -hydroxynorvaline and ϵ -hydroxynorleucine residues; canavine and canaline; γ -hydroxyornithine;

2-hexosaminic acids such as D-glucosaminic acid or D-galactosaminic acid;

α -Amino- β -thiols such as penicillamine, β -thiolnorvaline or β -thiolbutyrine;

Other sulfur containing amino acid residues including cysteine; homocystine, β -phenylmethionine, methionine, S-allyl-L-cysteine sulfoxide, 2-thiolhistidine, cystathionine, and thiol ethers of cysteine or homocysteine;

Phenylalanine, tryptophan and ring-substituted α -amino acids such as the phenyl- or cyclohexylamino acids α -aminophenylacetic acid, α -aminocyclohexylacetic acid and α -amino- β -cyclohexylpropionic acid; phenylalanine analogues and derivatives comprising aryl, lower alkyl, hydroxy, guanidino, oxyalkylether, nitro, sulfur or halo-substituted phenyl (e.g., tyrosine, methyltyrosine and *o*-chloro-, *p*-chloro-, 3,4-dichloro-, *o*-, *m*- or *p*-methyl-, 2,4,6-trimethyl-, 2-ethoxy-5-nitro-, 2-hydroxy-5-nitro- and *p*-nitro-phenylalanine); furyl-, thienyl-, pyridyl-, pyrimidinyl-, purinyl- or naphthyl-alanines; and tryptophan analogues and derivatives including kynurenine, 3-hydroxykynurenine, 2-hydroxytryptophan and 4-carboxytryptophan;

α -Amino substituted amino acids including sarcosine (N-methylglycine), N-benzylglycine, N-methylalanine, N-benzylalanine, N-methylphenylalanine, N-benzylphenylalanine, N-methylvaline and N-benzylvaline; and

α -Hydroxy and substituted α -hydroxy amino acids including serine, threonine, allothreonine, phosphoserine and phosphothreonine.

Polypeptides are polymers of amino acids in which a carboxyl group of one amino acid monomer is bonded to an amino or imino group of the next amino acid monomer by an amide bond. Polypeptides include dipeptides, low molecular weight polypeptides (about 1500-5000 MW) and proteins. Proteins optionally contain 3, 5, 10, 50, 75, 100 or more residues, and suitably are substantially sequence-homologous with human, animal, plant or microbial proteins. They include enzymes (e.g., hydrogen peroxidase) as well as immunogens such as KLH, or antibodies or proteins of any type against which one wishes to raise an immune response. The nature and identity of the polypeptide may vary widely.

The polypeptide amidates are useful as immunogens in raising antibodies against either the polypeptide (if it is not immunogenic in the animal to which it is administered) or against the epitopes on the remainder of the compound of this invention.

Antibodies capable of binding to the parental non-peptidyl compound are used to separate the parental compound from mixtures, for example in diagnosis or manufacturing of the parental compound. The conjugates of parental compound and polypeptide generally are more immunogenic than the polypeptides in closely homologous animals, and therefore make the polypeptide more immunogenic for facilitating raising antibodies against it. Accordingly, the polypeptide or protein may not need to be immunogenic in an animal typically used to raise antibodies, e.g., rabbit, mouse, horse, or rat, but the final product conjugate should be immunogenic in at least one of such animals. The polypeptide optionally contains a peptidolytic enzyme cleavage site at the peptide bond between the first and second residues adjacent to the acidic heteroatom. Such cleavage sites are flanked by enzymatic recognition structures, e.g. a particular sequence of residues recognized by a peptidolytic enzyme.

Peptidolytic enzymes for cleaving the polypeptide conjugates of this invention are well known, and in particular include carboxypeptidases. Carboxypeptidases digest polypeptides by removing C-terminal residues, and are specific in many instances for particular C-terminal sequences. Such enzymes and their substrate requirements in general are well known. For example, a dipeptide (having a given pair of residues and a free carboxyl terminus) is covalently bonded through its α -amino group to the phosphorus or carbon atoms of the compounds herein. In embodiments where W₁ is phosphonate it is expected that this peptide will be cleaved by the appropriate peptidolytic enzyme, leaving the carboxyl of the proximal amino acid residue to autocatalytically cleave the phosphonoamidate bond.

Suitable dipeptidyl groups (designated by their single letter code) are AA, AR, AN, AD, AC, AE, AQ, AG, AH, AI, AL, AK, AM, AF, AP, AS, AT, AW, AY, AV, RA, RR, RN, RD, RC, RE, RQ, RG, RH, RI, RL, RK, RM, RF, RP, RS, RT, RW, RY, RV, NA, NR, NN, ND, NC, NE, NQ, NG, NH, NI, NL, NK, NM, NF, NP, NS, NT, NW, NY, NV, DA, DR, DN, DD, DC, DE, DQ, DG, DH, DI, DL, DK, DM, DF, DP, DS, DT, DW, DY, DV, CA, CR, CN, CD, CC, CE, CQ, CG, CH, CI, CL, CK, CM, CF, CP, CS, CT, CW, CY, CV, EA, ER, EN, ED, EC, EE, EQ, EG, EH, EI, EL, EK, EM, EF, EP, ES, ET, EW, EY, EV, QA, QR, QN, QD, QC, QE, QQ, QG, QH, QI, QL, QK, QM, QF, QP, QS, QT, QW, QY, QV, GA, GR, GN, GD, GC, GE, GQ, GG, GH, GI, GL, GK, GM, GF, GP, GS, GT, GW, GY, GV, HA, HR, HN, HD, HC, HE, HQ, HG, HH, HI, HL, HK, HM, HF, HP, HS, HT, HW, HY, HV, IA, IR, IN, ID, IC, IE, IQ, IG, IH, IL, IK, IM, IF, IP, IS, IT, IW, IY, IV, LA, LR, LN, LD, LC, LE, LQ, LG, LH, LI, LL, LK, LM, LF, LP, LS, LT, LW, LY, LV, KA, KR,

KN, KD, KC, KE, KQ, KG, KH, KI, KL, KK, KM, KF, KP, KS, KT, KW, KY, KV, MA, MR, MN, MD, MC, ME, MQ, MG, MH, MI, ML, MK, MM, MF, MP, MS, MT, MW, MY, MV, FA, FR, FN, FD, FC, FE, FQ, FG, FH, FI, FL, FK, FM, FF, FP, FS, FT, FW, FY, FV, PA, PR, PN, PD, PC, PE, PQ, PG, PH, PI, PL, PK, PM, PF, PP, PS, PT, PW, PY, PV, SA, SR, SN, SD, SC, SE, SQ, SG, SH, SI, SL, SK, SM, SF, SP, SS, ST, SW, SY, SV, TA, TR, TN, TD, TC, TE, TQ, TG, TH, TI, TL, TK, TM, TF, TP, TS, TT, TW, TY, TV, WA, WR, WN, WD, WC, WE, WQ, WG, WH, WI, WL, WK, WM, WF, WP, WS, WT, WW, WY, WV, YA, YR, YN, YD, YC, YE, YQ, YG, YH, YI, YL, YK, YM, YF, YP, YS, YT, YW, YY, YV, VA, VR, VN, VD, VC, VE, VQ, VG, VH, VI, VL, VK, VM, VF, VP, VS, VT, VW, VY and VV.

Tripeptide residues are also useful as protecting groups. When a phosphonate is to be protected, the sequence $-X^4\text{-pro-X}^5-$ (where X^4 is any amino acid residue and X^5 is an amino acid residue, a carboxyl ester of proline, or hydrogen) will be cleaved by luminal carboxypeptidase to yield X^4 with a free carboxyl, which in turn is expected to autocatalytically cleave the phosphonoamidate bond. The carboxy group of X^5 optionally is esterified with benzyl.

Dipeptide or tripeptide species can be selected on the basis of known transport properties and/or susceptibility to peptidases that can affect transport to intestinal mucosal or other cell types. Dipeptides and tripeptides lacking an α -amino group are transport substrates for the peptide transporter found in brush border membrane of intestinal mucosal cells (Bai, J.P.F., (1992) *Pharm Res.* 9:969-978. Transport competent peptides can thus be used to enhance bioavailability of the amidate compounds. Di- or tripeptides having one or more amino acids in the D configuration are also compatible with peptide transport and can be utilized in the amidate compounds of this invention. Amino acids in the D configuration can be used to reduce the susceptibility of a di- or tripeptide to hydrolysis by proteases common to the brush border such as aminopeptidase N. In addition, di- or tripeptides alternatively are selected on the basis of their relative resistance to hydrolysis by proteases found in the lumen of the intestine. For example, tripeptides or polypeptides lacking asp and/or glu are poor substrates for aminopeptidase A, di- or tripeptides lacking amino acid residues on the N-terminal side of hydrophobic amino acids (leu, tyr, phe, val, trp) are poor substrates for endopeptidase, and peptides lacking a pro residue at the penultimate position at a free carboxyl terminus are poor substrates for carboxypeptidase P. Similar considerations can also

be applied to the selection of peptides that are either relatively resistant or relatively susceptible to hydrolysis by cytosolic, renal, hepatic, serum or other peptidases. Such poorly cleaved polypeptide amides are immunogens or are useful for bonding to proteins in order to prepare immunogens.

Prototype compounds contain at least one functional group capable of bonding to the phosphorus atom in the phosphonate moiety. The phosphonate candidate compounds are cleaved intracellularly after they have reached the desired site of action, e.g., inside a lymphoid cell. The mechanism by which this occurs is further described below in the examples. As noted, the free acid of the phosphonate is phosphorylated in the cell.

From the foregoing, it will be apparent that many different prototypes can be derivatized in accord with the present invention. Numerous such prototypes are specifically mentioned herein. However, it should be understood that the discussion of anti-HIV drug families and their specific members for derivatization according to this invention is not intended to be exhaustive, but merely illustrative.

When the prototype compound contains multiple reactive hydroxyl functions, a mixture of intermediates and final products may be obtained. In the unusual case in which all hydroxy groups are approximately equally reactive, there is not expected to be a single, predominant product, as each mono-substituted product will be obtained in approximately equal amounts, while a lesser amount of multiple-substituted candidate compound will also result. Generally speaking, however, one of the hydroxyl groups will be more susceptible to substitution than the other(s), e.g. a primary hydroxyl will be more reactive than a secondary hydroxyl, an unhindered hydroxyl will be more reactive than a hindered one. Consequently, the major product will be a mono-substituted one in which the most reactive hydroxyl has been derivatized while other mono-substituted and multiply-substituted products may be obtained as minor products.

Stereoisomers

The candidate compounds may have chiral centers, e.g. chiral carbon or phosphorus atoms. The compounds thus include racemic mixtures of all stereoisomers, including enantiomers, diastereomers, and atropisomers. In addition, the compounds include enriched or resolved optical isomers at any or all asymmetric, chiral atoms. In other words, the chiral centers apparent from the depictions are provided as the chiral isomers or racemic mixtures.

Both racemic and diastereomeric mixtures, as well as the individual optical isomers isolated or synthesized, substantially free of their enantiomeric or diastereomeric partners, are all suitable for use as candidate compounds. The racemic mixtures are separated into their individual, substantially optically pure isomers through well-known techniques such as, for example, the separation of diastereomeric salts formed with optically active adjuncts, e.g., acids or bases followed by conversion back to the optically active substances. In most instances, the desired optical isomer is synthesized by means of stereospecific reactions, beginning with the appropriate stereoisomer of the desired starting material.

The compounds can also exist as tautomeric isomers in certain cases. All though only one delocalized resonance structure may be depicted, all such forms are contemplated within the scope of the invention. For example, ene-amine tautomers can exist for purine, pyrimidine, imidazole, guanidine, amidine, and tetrazole systems and all their possible tautomeric forms are within the scope of the invention.

The optimal absolute configuration at the phosphorus atom for use in candidate compounds is that of GS-7340, depicted in the examples.

Salts and Hydrates

Any reference to any of the compounds of the invention also includes a reference to a physiologically acceptable salt thereof. Examples of physiologically acceptable salts of the compounds of the invention include salts derived from an appropriate base, such as an alkali metal (for example, sodium), an alkaline earth (for example, magnesium), ammonium and NX_4^+ (wherein X is C_1 – C_4 alkyl). Physiologically acceptable salts of a hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, benzoic, lactic, fumaric, tartaric, maleic, malonic, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids, such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids; and inorganic acids, such as hydrochloric, sulfuric, phosphoric and sulfamic acids.

Physiologically acceptable salts of a compound of an hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+ and NX_4^+ (wherein X is independently selected from H or a C_1 – C_4 alkyl group).

For therapeutic use, salts of active ingredients of the candidate compounds will be physiologically acceptable, i.e. they will be salts derived from a physiologically acceptable acid or base. However, salts of acids or bases which are not physiologically acceptable may also

find use, for example, in the preparation or purification of a physiologically acceptable compound. All salts, whether or not derived from a physiologically acceptable acid or base, are within the scope of the present invention.

Pharmaceutically acceptable non-toxic salts of candidate compounds containing, for example, Na^+ , Li^+ , K^+ , Ca^{+2} and Mg^{+2} , fall within the scope herein. Such salts may include those derived by combination of appropriate cations such as alkali and alkaline earth metal ions or ammonium and quaternary amino ions with an acid anion moiety, typically a carboxylic acid. Monovalent salts are preferred if a water soluble salt is desired.

Metal salts typically are prepared by reacting the metal hydroxide with a compound of this invention. Examples of metal salts which are prepared in this way are salts containing Li^+ , Na^+ , and K^+ . A less soluble metal salt can be precipitated from the solution of a more soluble salt by addition of the suitable metal compound.

In addition, salts may be formed from acid addition of certain organic and inorganic acids, e.g., HCl , HBr , H_2SO_4 , H_3PO_4 or organic sulfonic acids, to basic centers, typically amines, or to acidic groups. Finally, it is to be understood that the compositions herein comprise compounds of the invention in their un-ionized, as well as zwitterionic form, and combinations with stoichiometric amounts of water as in hydrates.

Salts of the candidate compounds with amino acids also fall within the scope of this invention. Any of the amino acids described above are suitable, especially the naturally-occurring amino acids found as protein components, although the amino acid typically is one bearing a side chain with a basic or acidic group, e.g., lysine, arginine or glutamic acid, or a neutral group such as glycine, serine, threonine, alanine, isoleucine, or leucine.

Methods for Assay of Anti-HIV Activity

The anti-HIV activity of a candidate compound is assayed by any method heretofore known for determining inhibition of growth, replication, or other characteristic of HIV infection, including direct and indirect methods of detecting HIV activity. Quantitative, qualitative, and semiquantitative methods of determining HIV activity are all contemplated. Typically any one of the *in vitro* or cell culture screening methods known to the art are employed, as are clinical trials in humans, studies in animal models (SIV), and the like. In screening candidate compounds it should be kept in mind that the results of enzyme assays

may not correlate with cell culture assays. Thus, a cell based assay is often the primary screening tool. Candidate compounds having an *in vitro* K_i (inhibitory constant) of less than about 5×10^{-6} M, typically less than about 1×10^{-7} M and preferably less than about 5×10^{-8} M are preferred for *in vivo* development, but the analytical point of selection of a candidate compound for further development is essentially a matter of choice.

Pharmaceutical Formulations

Candidate compounds selected for further development *in vivo* are formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. All formulations will optionally contain excipients such as those set forth in the "Handbook of Pharmaceutical Excipients" (1986). Excipients include ascorbic acid and other antioxidants, chelating agents such as EDTA, carbohydrates such as dextrin, hydroxyalkylcellulose, hydroxyalkylmethylcellulose, stearic acid and the like. The pH of the formulations ranges from about 3 to about 11, but is ordinarily about 7 to 10.

While it is possible for the active ingredients to be administered alone it may be preferable to present them as pharmaceutical formulations. The formulations, both for veterinary and for human use, of the invention comprise at least one active ingredient, as above defined, together with one or more acceptable carriers therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and physiologically innocuous to the recipient thereof.

The formulations include those suitable for the foregoing administration routes. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Techniques and formulations generally are found in Remington's Pharmaceutical Sciences (Mack Publishing Co., Easton, PA). Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations of candidate compounds suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet is made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, preservative, surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent. The tablets may optionally be coated or scored and optionally are formulated so as to provide slow or controlled release of the active ingredient therefrom.

For infections of the eye or other external tissues e.g. mouth and skin, the formulations are preferably applied as a topical ointment or cream containing the active ingredient(s) in an amount of, for example, 0.075 to 20% w/w (including active ingredient(s) in a range between 0.1% and 20% in increments of 0.1% w/w such as 0.6% w/w, 0.7% w/w, etc.), preferably 0.2 to 15% w/w and most preferably 0.5 to 10% w/w. When formulated in an ointment, the active ingredients may be employed with either a paraffinic or a water-miscible ointment base. Alternatively, the active ingredients may be formulated in a cream with an oil-in-water cream base.

If desired, the aqueous phase of the cream base may include, for example, at least 30% w/w of a polyhydric alcohol, i.e. an alcohol having two or more hydroxyl groups such as propylene glycol, butane 1,3-diol, mannitol, sorbitol, glycerol and polyethylene glycol (including PEG 400) and mixtures thereof. The topical formulations may desirably include a compound which enhances absorption or penetration of the active ingredient through the skin or other affected areas. Examples of such dermal penetration enhancers include dimethyl sulphoxide and related analogs.

The oily phase of the emulsions of this invention may be constituted from known ingredients in a known manner. While the phase may comprise merely an emulsifier

(otherwise known as an emulgent), it desirably comprises a mixture of at least one emulsifier with a fat or an oil or with both a fat and an oil. Preferably, a hydrophilic emulsifier is included together with a lipophilic emulsifier which acts as a stabilizer. It is also preferred to include both an oil and a fat. Together, the emulsifier(s) with or without stabilizer(s) make up the so-called emulsifying wax, and the wax together with the oil and fat make up the so-called emulsifying ointment base which forms the oily dispersed phase of the cream formulations.

Emulgents and emulsion stabilizers suitable for use in the formulation of the invention include Tween[®] 60, Span[®] 80, cetostearyl alcohol, benzyl alcohol, myristyl alcohol, glyceryl mono-stearate and sodium lauryl sulfate.

The choice of suitable oils or fats for the formulation is based on achieving the desired cosmetic properties. The cream should preferably be a non-greasy, non-staining and washable product with suitable consistency to avoid leakage from tubes or other containers. Straight or branched chain, mono- or dibasic alkyl esters such as di-isoadipate, isocetyl stearate, propylene glycol diester of coconut fatty acids, isopropyl myristate, decyl oleate, isopropyl palmitate, butyl stearate, 2-ethylhexyl palmitate or a blend of branched chain esters known as Crodamol CAP may be used, the last three being preferred esters. These may be used alone or in combination depending on the properties required. Alternatively, high melting point lipids such as white soft paraffin and/or liquid paraffin or other mineral oils are used.

Pharmaceutical formulations according to the present invention comprise a combination according to the invention together with one or more pharmaceutically acceptable carriers or excipients and optionally other therapeutic agents. Pharmaceutical formulations containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets containing the active ingredient in admixture with non-toxic pharmaceutically acceptable excipient which are suitable for manufacture of tablets are acceptable. These excipients may be, for example, inert diluents, such as calcium or sodium carbonate, lactose, calcium or sodium phosphate; granulating and disintegrating agents, such as maize starch, or alginate acid;

binding agents, such as starch, gelatin or acacia; and lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be uncoated or may be coated by known techniques including microencapsulation to delay disintegration and adsorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

Formulations for oral use may be also presented as hard gelatin capsules where the active ingredient is mixed with an inert solid diluent, for example calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, such as peanut oil, liquid paraffin or olive oil.

Aqueous suspensions of the invention contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropyl methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia, and dispersing or wetting agents such as a naturally occurring phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate, one or more coloring agents, one or more flavoring agents and one or more sweetening agents, such as sucrose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oral suspensions may contain a thickening agent, such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an antioxidant such as ascorbic acid.

Dispersible powders and granules of the invention suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a

dispersing or wetting agent, a suspending agent, and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those disclosed above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the candidate compounds may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable emulsifying agents include naturally-occurring gums, such as gum acacia and gum tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and condensation products of these partial esters with ethylene oxide, such as polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and flavoring agents. Syrups and elixirs may be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a flavoring or a coloring agent.

The pharmaceutical compositions of the candidate compounds may be in the form of a sterile injectable preparation, such as a sterile injectable aqueous or oleaginous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain approximately 1 to 1000 mg of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions (weight:weight). The pharmaceutical

composition can be prepared to provide easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion may contain from about 3 to 500 μg of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

Formulations suitable for topical administration to the eye also include eye drops wherein the active ingredient is dissolved or suspended in a suitable carrier, especially an aqueous solvent for the active ingredient. The active ingredient is preferably present in such formulations in a concentration of 0.5 to 20%, advantageously 0.5 to 10% particularly about 1.5% w/w.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavored basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising for example cocoa butter or a salicylate.

Formulations suitable for intrapulmonary or nasal administration have a particle size for example in the range of 0.1 to 500 microns (including particle sizes in a range between 0.1 and 500 microns in increments microns such as 0.5, 1, 30 microns, 35 microns, etc.), which is administered by rapid inhalation through the nasal passage or by inhalation through the mouth so as to reach the alveolar sacs. Suitable formulations include aqueous or oily solutions of the active ingredient. Formulations suitable for aerosol or dry powder administration may be prepared according to conventional methods and may be delivered with other therapeutic agents such as compounds heretofore used in the treatment or prophylaxis of HIV infections as described below.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents.

The formulations are presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions are prepared from sterile powders, granules and tablets of the kind previously described. Preferred unit dosage formulations are those containing a daily dose or unit daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the ingredients particularly mentioned above the formulations of candidate compounds may include other agents conventional in the art having regard to the type of formulation in question, for example those suitable for oral administration may include flavoring agents.

The invention further provides veterinary compositions comprising at least one active ingredient as above defined together with a veterinary carrier therefor.

Veterinary carriers are materials useful for the purpose of administering the composition and may be solid, liquid or gaseous materials which are otherwise inert or acceptable in the veterinary art and are compatible with the active ingredient. These veterinary compositions may be administered orally, parenterally or by any other desired route.

Compounds of the invention are used to provide controlled release pharmaceutical formulations containing as active ingredient one or more compounds of the invention ("controlled release formulations") in which the release of the active ingredient are controlled and regulated to allow less frequency dosing or to improve the pharmacokinetic or toxicity profile of a given active ingredient.

An effective dose of candidate compound depends at least on the nature of the

condition being treated, toxicity, whether the compound is being used prophylactically (lower doses) or against an active HIV infection, the method of delivery, and the pharmaceutical formulation, and will be determined by the clinician using conventional dose escalation studies. It can be expected to be from about 0.0001 to about 100 mg/kg body weight per day. Typically, from about 0.01 to about 10 mg/kg body weight per day. More typically, from about .01 to about 5 mg/kg body weight per day. More typically, from about .05 to about 0.5 mg/kg body weight per day. For example, the daily candidate dose for an adult human of approximately 70 kg body weight will range from 1 mg to 1000 mg, preferably between 5 mg and 500 mg, and may take the form of single or multiple doses.

Routes of Administration

One or more candidate compounds (herein referred to as the active ingredients) are administered by any route appropriate to the condition to be treated. Suitable routes include oral, rectal, nasal, topical (including buccal and sublingual), vaginal and parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural), and the like. It will be appreciated that the preferred route may vary with for example the condition of the recipient. An advantage of the compounds of this invention is that they are orally bioavailable and can be dosed orally.

Combination Therapy

Candidate compound are also used in combination with other active ingredients. Such combinations are selected based on the condition to be treated, cross-reactivities of ingredients and pharmaco- compounds. Other active ingredients include adefovir dipivoxil and/or any other product currently marketed for therapy of HIV infection. It is also possible to combine any compound of the invention with one or more other active ingredients in a unitary dosage form for simultaneous or sequential administration to an HIV infected patient. The combination therapy may be administered as a simultaneous or sequential regimen. When administered sequentially, the combination may be administered in two or more administrations. Second and third active ingredients in the combination may have anti-HIV activity and include HIV.

The combination therapy may be synergistic, i.e. the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are:

(1) co-formulated and administered or delivered simultaneously in a combined formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect may be attained when the compounds are administered or delivered sequentially, e.g. in separate tablets, pills or capsules, or by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together. A synergistic anti-viral effect denotes an antiviral effect which is greater than the predicted purely additive effects of the individual compounds of the combination.

Metabolites of the Candidate Compounds

The candidate compounds are metabolized *in vivo*. In particular, the group R^x is hydrolytically cleaved to produce a charged metabolite, and in some cases the substituents on the phosphonate such as $-Y^2[P((=Y^1)(Y^2))_m R^x]_2$ are hydrolyzed as well. An example showing exemplary metabolites is found in the examples herein. While this example is concerned with the metabolites of GS-7340, a nucleotide analogue, the metabolic changes to be found with candidate compounds are believed to be substantially the same at the phosphonate substituent. This charged metabolite functions as an intracellular depot form of the candidate. However, other changes may result for example from the oxidation, reduction, hydrolysis, amidation, esterification and the like of the administered compound, primarily due to enzymatic processes. Accordingly, candidate compounds include metabolites of candidate compounds produced by a process comprising contacting a compound of this invention with a mammal for a period of time sufficient to yield a metabolic product thereof. Such products typically are identified by preparing a radiolabelled (e.g. C^{14} or H^3) compound of the invention, administering it parenterally in a detectable dose (e.g. greater than about 0.5 mg/kg) to an animal such as rat, mouse, guinea pig, monkey, or to man, allowing sufficient time for metabolism to occur (typically about 30 seconds to 30 hours) and isolating its conversion products from the urine, blood or other biological samples. These products are easily isolated since they are labeled (others are isolated by the use of antibodies capable of binding epitopes surviving in the metabolite). The metabolite structures are determined in conventional fashion, e.g. by MS or NMR analysis. In general, analysis of metabolites is done in the same way as conventional drug metabolism studies well-known to those skilled in the art. The conversion products, so long as they are not otherwise found *in vivo*, are useful in diagnostic assays for therapeutic dosing of the candidate compounds even if they possess no HIV inhibitory activity of their own.

Recipes and methods for determining stability of compounds in surrogate gastrointestinal secretions are known. Compounds are defined herein as stable in the gastrointestinal tract where less than about 50 mole percent of the protected groups are deprotected in surrogate intestinal or gastric juice upon incubation for 1 hour at 37 °C. Simply because the compounds are stable to the gastrointestinal tract does not mean that they cannot be hydrolyzed *in vivo*. The phosphonate prodrugs of the invention typically will be stable in the digestive system but are substantially hydrolyzed to the parental drug in the digestive lumen, liver or other metabolic organ, or within cells in general.

Exemplary Methods of Making Candidate Compounds.

The candidate compounds are prepared by any of the applicable techniques of organic synthesis. Many such techniques are well known in the art. However, many of the known techniques are elaborated in "Compendium of Organic Synthetic Methods" (John Wiley & Sons, New York), Vol. 1, Ian T. Harrison and Shuyen Harrison, 1971; Vol. 2, Ian T. Harrison and Shuyen Harrison, 1974; Vol. 3, Louis S. Hegedus and Leroy Wade, 1977; Vol. 4, Leroy G. Wade, jr., 1980; Vol. 5, Leroy G. Wade, Jr., 1984; and Vol. 6, Michael B. Smith; as well as March, J., "Advanced Organic Chemistry, Third Edition", (John Wiley & Sons, New York, 1985), "Comprehensive Organic Synthesis. Selectivity, Strategy & Efficiency in Modern Organic Chemistry. In 9 Volumes", Barry M. Trost, Editor-in-Chief (Pergamon Press, New York, 1993 printing).

Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; US Patent No. 5663159.

In general, synthesis of phosphonate esters is achieved by coupling a nucleophile amine or alcohol with the corresponding activated phosphonate electrophilic precursor. For example, chlorophosphonate addition on to 5'-hydroxy of nucleoside is a well known method for preparation of nucleoside phosphate monoesters. The activated precursor can be prepared by several well known methods. Chlorophosphonates useful for synthesis of the prodrugs are prepared from the substituted-1,3-propanediol (Wissner, et al, (1992) *J. Med Chem.* 35:1650). Chlorophosphonates are made by oxidation of the corresponding chlorophospholanes (Anderson, et al, (1984) *J. Org. Chem.* 49:1304) which are obtained by reaction of the substituted diol with phosphorus trichloride. Alternatively, the chlorophosphonate agent is made by treating substituted-1,3-diols with phosphorus oxychloride (Patois, et al, (1990) *J.*

Chem. Soc. Perkin Trans. I, 1577). Chlorophosphonate species may also be generated in situ from corresponding cyclic phosphites (Silverburg, et al., (1996) *Tetrahedron lett.*, 37:771-774), which in turn can be either made from chlorophospholane or phosphoramidate intermediate. The phosphorofluoridate intermediate prepared either from pyrophosphate or phosphoric acid may also act as precursor in preparation of cyclic prodrugs (Watanabe et al., (1988) *Tetrahedron lett.*, 29:5763-66).

Candidate compounds comprising a prodrug functionality may also be prepared from the free acid by Mitsunobu reactions (Mitsunobu, (1981) *Synthesis*, 1; Campbell, (1992) *J. Org. Chem.*, 52:6331), and other acid coupling reagents including, but not limited to, carbodiimides (Alexander, et al, (1994) *Collect. Czech. Chem. Commun.* 59:1853; Casara, et al, (1992) *Bioorg. Med. Chem. Lett.*, 2:145; Ohashi, et al, (1988) *Tetrahedron Lett.*, 29:1189), and benzotriazolyloxytris-(dimethylamino)phosphonium salts (Campagne, et al, (1993) *Tetrahedron Lett.*, 34:6743).

Aryl halides undergo Ni^{+2} catalyzed reaction with phosphite derivatives to give aryl phosphonate containing compounds (Balthazar, et al (1980) *J. Org. Chem.* 45:5425). Phosphonates may also be prepared from the chlorophosphonate in the presence of a palladium catalyst using aromatic triflates (Petrakis, et al, (1987) *J. Am. Chem. Soc.* 109:2831; Lu, et al, (1987) *Synthesis*, 726). In another method, aryl phosphonate esters are prepared from aryl phosphates under anionic rearrangement conditions (Melvin (1981) *Tetrahedron Lett.* 22:3375; Casteel, et al, (1991) *Synthesis*, 691). N-Alkoxy aryl salts with alkali metal derivatives of cyclic alkyl phosphonate provide general synthesis for heteroaryl-2-phosphonate linkers (Redmore (1970) *J. Org. Chem.* 35:4114). These above mentioned methods can also be extended to compounds where the W^5 group is a heterocycle. Cyclic-1,3-propanyl prodrugs of phosphonates are also synthesized from phosphonic diacids and substituted propane-1,3-diols using a coupling reagent such as 1,3-dicyclohexylcarbodiimide (DCC) in presence of a base (e.g., pyridine). Other carbodiimide based coupling agents like 1,3-disopropylcarbodiimide or water soluble reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) can also be utilized for the synthesis of cyclic phosphonate prodrugs.

The carbamoyl group may be formed by reaction of a hydroxy group according to the methods known in the art, including the teachings of Ellis, U.S. 2002/0103378 A1 and Hajima, U.S. 6,018,049.

A number of exemplary methods for the preparation of the candidate compounds are provided below. These methods are intended to illustrate the nature of such preparations and do not limit the scope of this invention. Many of the compounds set forth below have been screened and demonstrated to have anti-HIV activity. In view of this these compounds are no longer candidate compounds for use in the screening method of this invention. However, they are illustrative of the manner in which the artisan can substitute prototype compounds with A³ in various ways. In addition, taken cumulatively, they are illustrative of the typical component candidate compounds to be found in a screening library.

Generally, the reaction conditions such as temperature, reaction time, solvents, work-up procedures, and the like, will be those common in the art for the particular reaction to be performed. The cited reference material, together with material cited therein, contains detailed descriptions of such conditions. Typically the temperatures will be -100°C to 200°C, solvents will be aprotic or protic, and reaction times will be 10 seconds to 10 days. Work-up typically consists of quenching any unreacted reagents followed by partition between a water/organic layer system (extraction) and separating the layer containing the product.

Oxidation and reduction reactions are typically carried out at temperatures near room temperature (about 20 °C), although for metal hydride reductions frequently the temperature is reduced to 0 °C to -100 °C, solvents are typically aprotic for reductions and may be either protic or aprotic for oxidations. Reaction times are adjusted to achieve desired conversions.

Condensation reactions are typically carried out at temperatures near room temperature, although for non-equilibrating, kinetically controlled condensations reduced temperatures (0 °C to -100 °C) are also common. Solvents can be either protic (common in equilibrating reactions) or aprotic (common in kinetically controlled reactions).

Standard synthetic techniques such as azeotropic removal of reaction by-products and use of anhydrous reaction conditions (e.g. inert gas environments) are common in the art and will be applied when applicable.

Schemes

General aspects of these exemplary methods are described below and in the Examples. Each of the products of the following processes are optionally separated, isolated, and/or

purified prior to its use in subsequent processes.

The terms "treated", "treating", "treatment", and the like, mean contacting, mixing, reacting, allowing to react, bringing into contact, and other terms common in the art for indicating that one or more chemical entities is treated in such a manner as to convert it to one or more other chemical entities. This means that "treating compound one with compound two" is synonymous with "allowing compound one to react with compound two", "contacting compound one with compound two", "reacting compound one with compound two", and other expressions common in the art of organic synthesis for reasonably indicating that compound one was "treated", "reacted", "allowed to react", etc., with compound two.

"Treating" indicates the reasonable and usual manner in which organic chemicals are allowed to react. Normal concentrations (0.01M to 10M, typically 0.1M to 1M), temperatures (-100 °C to 250 °C, typically -78 °C to 150 °C, more typically -78 °C to 100 °C, still more typically 0 °C to 100 °C), reaction vessels (typically glass, plastic, metal), solvents, pressures, atmospheres (typically air for oxygen and water insensitive reactions or nitrogen or argon for oxygen or water sensitive), etc., are intended unless otherwise indicated. The knowledge of similar reactions known in the art of organic synthesis are used in selecting the conditions and apparatus for "treating" in a given process. In particular, one of ordinary skill in the art of organic synthesis selects conditions and apparatus reasonably expected to successfully carry out the chemical reactions of the described processes based on the knowledge in the art.

Modifications of each of the exemplary schemes above and in the examples (hereafter "exemplary schemes") leads to various analogs of the candidate compounds. The above cited citations describing suitable methods of organic synthesis are applicable to such modifications.

In each of the exemplary schemes it may be advantageous to separate reaction products from one another and/or from starting materials. The desired products of each step or series of steps is separated and/or purified (hereinafter separated) to the desired degree of homogeneity by the techniques common in the art. Typically such separations involve multiphase extraction, crystallization from a solvent or solvent mixture, distillation, sublimation, or chromatography. Chromatography can involve any number of methods including, for example: reverse-phase and normal phase; size exclusion; ion exchange; high,

medium, and low pressure liquid chromatography methods and apparatus; small scale analytical; simulated moving bed (SMB) and preparative thin or thick layer chromatography, as well as techniques of small scale thin layer and flash chromatography.

Another class of separation methods involves treatment of a mixture with a reagent selected to bind to or render otherwise separable a desired product, unreacted starting material, reaction by product, or the like. Such reagents include adsorbents such as activated carbon, molecular sieves, ion exchange media, or the like. Alternatively, the reagents can be acids in the case of a basic material, bases in the case of an acidic material, binding reagents such as antibodies, binding proteins, selective chelators such as crown ethers, liquid/liquid ion extraction reagents (LIX), or the like.

Selection of appropriate methods of separation depends on the nature of the materials involved. These include boiling point and molecular weight in distillation and sublimation, presence or absence of polar functional groups in chromatography, stability of materials in acidic and basic media in multiphase extraction, and the like. One skilled in the art will apply techniques most likely to achieve the desired separation.

A single stereoisomer, e.g. an enantiomer, substantially free of its stereoisomer may be obtained by resolution of the racemic mixture using a method such as formation of diastereomers using optically active resolving agents ("Stereochemistry of Carbon Compounds," (1962) by E. L. Eliel, McGraw Hill; Lochmuller, C. H., (1975) *J. Chromatogr.*, 113:(3) 283-302). Racemic mixtures of chiral compounds of the invention can be separated and isolated by any suitable method, including: (1) formation of ionic, diastereomeric salts with chiral compounds and separation by fractional crystallization or other methods, (2) formation of diastereomeric compounds with chiral derivatizing reagents, separation of the diastereomers, and conversion to the pure stereoisomers, and (3) separation of the substantially pure or enriched stereoisomers directly under chiral conditions.

Under method (1), diastereomeric salts can be formed by reaction of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, α -methyl- β -phenylethylamine (amphetamine), and the like with asymmetric compounds bearing acidic functionality, such as carboxylic acid and sulfonic acid. The diastereomeric salts may be induced to separate by fractional crystallization or ionic chromatography. For separation of the optical isomers of

amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

Alternatively, by method (2), the substrate to be resolved is reacted with one enantiomer of a chiral compound to form a diastereomeric pair (Eliel, E. and Wilen, S. (1994) *Stereochemistry of Organic Compounds*, John Wiley & Sons, Inc., p. 322). Diastereomeric compounds can be formed by reacting asymmetric compounds with enantiomerically pure chiral derivatizing reagents, such as menthyl derivatives, followed by separation of the diastereomers and hydrolysis to yield the free, enantiomerically enriched xanthene. A method of determining optical purity involves making chiral esters, such as a menthyl ester, e.g. (-)-menthyl chloroformate in the presence of base, or Mosher ester, α -methoxy- α -(trifluoromethyl)phenyl acetate (Jacob III. (1982) *J. Org. Chem.* 47:4165), of the racemic mixture, and analyzing the NMR spectrum for the presence of the two atropisomeric diastereomers. Stable diastereomers of atropisomeric compounds can be separated and isolated by normal- and reverse-phase chromatography following methods for separation of atropisomeric naphthyl-isoquinolines (Hoye, T., WO 96/15111). By method (3), a racemic mixture of two enantiomers can be separated by chromatography using a chiral stationary phase ("Chiral Liquid Chromatography" (1989) W. J. Lough, Ed. Chapman and Hall, New York; Okamoto, (1990) *J. of Chromatogr.* 513:375-378). Enriched or purified enantiomers can be distinguished by methods used to distinguish other chiral molecules with asymmetric carbon atoms, such as optical rotation and circular dichroism.

The articles "and" and "or" shall be construed as meaning "and/or" unless otherwise required by context or usage. Use of "and/or" herein shall not be construed as foreclosing "and/or" when only "and" or "or" are employed in other circumstances.

This invention includes all novel and unobvious compounds disclosed herein, whether or not such compounds are described in the context of methods or other disclosure and whether or not such compounds are claimed upon filing or are set forth in the summary of invention.

The invention has been described in detail sufficient to allow one of ordinary skill in the art to make and use the subject matter of the following examples. It is apparent that certain modifications of the methods and compositions of the following examples can be made within

the scope and spirit of the invention.

Examples General Section

Some Examples have been performed multiple times. In repeated Examples, reaction conditions such as time, temperature, concentration and the like, and yields were within normal experimental ranges. In repeated Examples where significant modifications were made, these have been noted where the results varied significantly from those described. In Examples where different starting materials were used, these are noted. When the repeated Examples refer to a "corresponding" analog of a compound, such as a "corresponding ethyl ester", this intends that an otherwise present group, in this case typically a methyl ester, is taken to be the same group modified as indicated.

Exemplary Methods of Making the Compounds of the Invention.

The invention provides many methods of making the compositions of the invention. The compositions are prepared by any of the applicable techniques of organic synthesis. Many such techniques are well known in the art. Such as those elaborated in "Compendium of Organic Synthetic Methods" (John Wiley & Sons, New York), Vol. 1, Ian T. Harrison and Shuyen Harrison, 1971; Vol. 2, Ian T. Harrison and Shuyen Harrison, 1974; Vol. 3, Louis S. Hegedus and Leroy Wade, 1977; Vol. 4, Leroy G. Wade, jr., 1980; Vol. 5, Leroy G. Wade, Jr., 1984; and Vol. 6, Michael B. Smith; as well as March, J., "Advanced Organic Chemistry, Third Edition", (John Wiley & Sons, New York, 1985), "Comprehensive Organic Synthesis. Selectivity, Strategy & Efficiency in Modern Organic Chemistry. In 9 Volumes", Barry M. Trost, Editor-in-Chief (Pergamon Press, New York, 1993 printing).

Dialkyl phosphonates may be prepared according to the methods of: Quast et al (1974) *Synthesis* 490; Stowell et al (1990) *Tetrahedron Lett.* 3261; US Patent No. 5663159.

In general, synthesis of phosphonate esters is achieved by coupling a nucleophile amine or alcohol with the corresponding activated phosphonate electrophilic precursor for example, Chlorophosphonate addition on to 5'-hydroxy of nucleoside is a well known method for preparation of nucleoside phosphate monoesters. The activated precursor can be prepared by several well known methods. Chlorophosphonates useful for synthesis of the prodrugs are prepared from the substituted-1,3-propanediol (Wissner, et al, (1992) *J. Med Chem.* 35:1650). Chlorophosphonates are made by oxidation of the corresponding chlorophospholanes (Anderson, et al, (1984) *J. Org. Chem.* 49:1304) which are obtained by reaction of the substituted diol with phosphorus trichloride. Alternatively, the chlorophosphonate agent is

made by treating substituted-1,3-diols with phosphorusoxychloride (Patois, et al, (1990) *J. Chem. Soc. Perkin Trans. I*, 1577). Chlorophosphonate species may also be generated in situ from corresponding cyclic phosphites (Silverburg, et al., (1996) *Tetrahedron lett.*, 37:771-774), which in turn can be either made from chlorophospholane or phosphoramidate intermediate. Phosphorofluoridate intermediate prepared either from pyrophosphate or phosphoric acid may also act as precursor in preparation of cyclic prodrugs (Watanabe et al., (1988) *Tetrahedron lett.*, 29:5763-66). Caution: fluorophosphonate compounds may be highly toxic!

Schemes and Examples

General aspects of these exemplary methods are described below and in the Examples. Each of the products of the following processes is optionally separated, isolated, and/or purified prior to its use in subsequent processes.

A number of exemplary methods for the preparation of the compositions of the invention are provided below. These methods are intended to illustrate the nature of such preparations are not intended to limit the scope of applicable methods.

The terms "treated", "treating", "treatment", and the like, mean contacting, mixing, reacting, allowing to react, bringing into contact, and other terms common in the art for indicating that one or more chemical entities is treated in such a manner as to convert it to one or more other chemical entities. This means that "treating compound one with compound two" is synonymous with "allowing compound one to react with compound two", "contacting compound one with compound two", "reacting compound one with compound two", and other expressions common in the art of organic synthesis for reasonably indicating that compound one was "treated", "reacted", "allowed to react", etc., with compound two.

"Treating" indicates the reasonable and usual manner in which organic chemicals are allowed to react. Normal concentrations (0.01M to 10M, typically 0.1M to 1M), temperatures (-100°C to 250°C, typically -78°C to 150°C, more typically -78°C to 100°C, still more typically 0°C to 100°C), reaction vessels (typically glass, plastic, metal), solvents, pressures, atmospheres (typically air for oxygen and water insensitive reactions or nitrogen or argon for oxygen or water sensitive), etc., are intended unless otherwise indicated. The knowledge of similar reactions known in the art of organic synthesis are used in selecting the conditions and apparatus for "treating" in a given process. In particular, one of ordinary skill in the art of organic synthesis selects conditions and apparatus reasonably expected to successfully carry out the chemical reactions of the described processes based on the knowledge in the art.

Modifications of each of the exemplary schemes above and in the examples (hereafter "exemplary schemes") leads to various analogs of the specific exemplary materials produce. The above cited citations describing suitable methods of organic synthesis are applicable to such modifications.

In each of the exemplary schemes it may be advantageous to separate reaction products from one another and/or from starting materials. The desired products of each step or series of steps is separated and/or purified (hereinafter separated) to the desired degree of homogeneity by the techniques common in the art. Typically such separations involve multiphase extraction, crystallization from a solvent or solvent mixture, distillation, sublimation, or chromatography. Chromatography can involve any number of methods including, for example: reverse-phase and normal phase; size exclusion; ion exchange; high, medium, and low pressure liquid chromatography methods and apparatus; small scale analytical; simulated moving bed (SMB) and preparative thin or thick layer chromatography, as well as techniques of small scale thin layer and flash chromatography.

Another class of separation methods involves treatment of a mixture with a reagent selected to bind to or render otherwise separable a desired product, unreacted starting material, reaction by product, or the like. Such reagents include adsorbents or absorbents such as activated carbon, molecular sieves, ion exchange media, or the like. Alternatively, the reagents can be acids in the case of a basic material, bases in the case of an acidic material, binding reagents such as antibodies, binding proteins, selective chelators such as crown ethers, liquid/liquid ion extraction reagents (LIX), or the like.

Selection of appropriate methods of separation depends on the nature of the materials involved. For example, boiling point, and molecular weight in distillation and sublimation, presence or absence of polar functional groups in chromatography, stability of materials in acidic and basic media in multiphase extraction, and the like. One skilled in the art will apply techniques most likely to achieve the desired separation.

A single stereoisomer, e.g. an enantiomer, substantially free of its stereoisomer may be obtained by resolution of the racemic mixture using a method such as formation of diastereomers using optically active resolving agents ("Stereochemistry of Carbon Compounds," (1962) by E. L. Eliel, McGraw Hill; Lochmuller, C. H., (1975) *J. Chromatogr.*, 113:(3) 283-302). Racemic mixtures of chiral compounds of the invention can be separated and isolated by any suitable method, including: (1) formation of ionic, diastereomeric salts with chiral compounds and separation by fractional crystallization or other methods, (2) formation of diastereomeric compounds with chiral derivatizing reagents, separation of the

diastereomers, and conversion to the pure stereoisomers, and (3) separation of the substantially pure or enriched stereoisomers directly under chiral conditions.

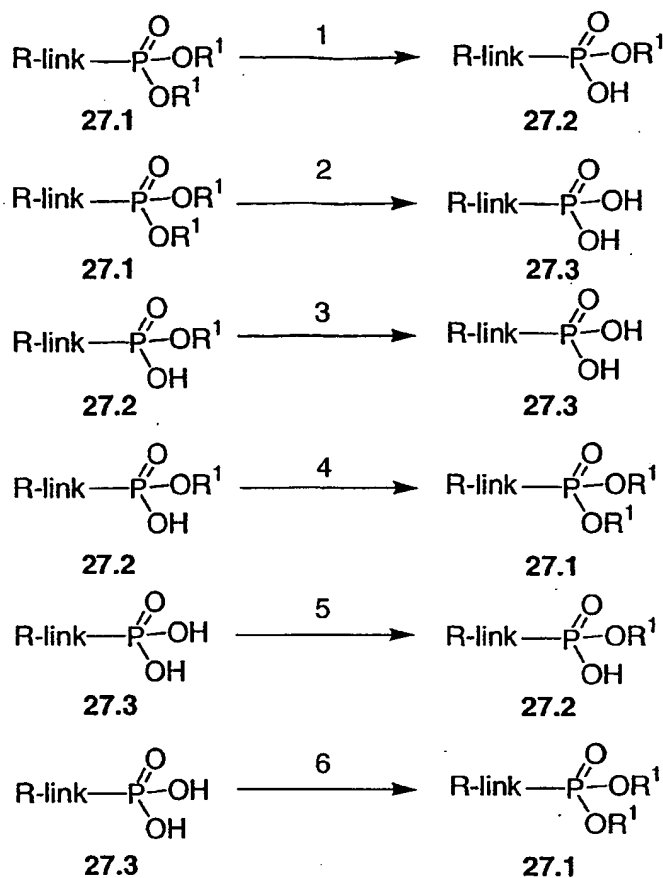
Under method (1), diastereomeric salts can be formed by reaction of enantiomerically pure chiral bases such as brucine, quinine, ephedrine, strychnine, α -methyl- β -phenylethylamine (amphetamine), and the like with asymmetric compounds bearing acidic functionality, such as carboxylic acid and sulfonic acid. The diastereomeric salts may be induced to separate by fractional crystallization or ionic chromatography. For separation of the optical isomers of amino compounds, addition of chiral carboxylic or sulfonic acids, such as camphorsulfonic acid, tartaric acid, mandelic acid, or lactic acid can result in formation of the diastereomeric salts.

Alternatively, by method (2), the substrate to be resolved is reacted with one enantiomer of a chiral compound to form a diastereomeric pair (Elie, E. and Wilen, S. (1994) *Stereochemistry of Organic Compounds*, John Wiley & Sons, Inc., p. 322). Diastereomeric compounds can be formed by reacting asymmetric compounds with enantiomerically pure chiral derivatizing reagents, such as menthyl derivatives, followed by separation of the diastereomers and hydrolysis to yield the free, enantiomerically enriched xanthene. A method of determining optical purity involves making chiral esters, such as a menthyl ester, e.g. (-) menthyl chloroformate in the presence of base, or Mosher ester, α -methoxy- α -(trifluoromethyl)phenyl acetate (Jacob III. (1982) *J. Org. Chem.* 47:4165), of the racemic mixture, and analyzing the NMR spectrum for the presence of the two atropisomeric diastereomers. Stable diastereomers of atropisomeric compounds can be separated and isolated by normal- and reverse-phase chromatography following methods for separation of atropisomeric naphthyl-isoquinolines (Hoye, T., WO 96/15111). By method (3), a racemic mixture of two enantiomers can be separated by chromatography using a chiral stationary phase ("Chiral Liquid Chromatography" (1989) W. J. Lough, Ed. Chapman and Hall, New York; Okamoto, (1990) *J. of Chromatogr.* 513:375-378). Enriched or purified enantiomers can be distinguished by methods used to distinguish other chiral molecules with asymmetric carbon atoms, such as optical rotation and circular dichroism.

All literature and patent citations above are hereby expressly incorporated by reference at the locations of their citation. Specifically cited sections or pages of the above cited works are incorporated by reference with specificity. The invention has been described in detail sufficient to allow one of ordinary skill in the art to make and use the subject matter of the following Embodiments. It is apparent that certain modifications of the methods and compositions of the following Embodiments can be made within the scope and spirit of the

invention.

Scheme A



Scheme A shows the general interconversions of certain phosphonate compounds: acids $\text{-P}(\text{O})(\text{OH})_2$; mono-esters $\text{-P}(\text{O})(\text{OR}_1)(\text{OH})$; and diesters $\text{-P}(\text{O})(\text{OR}_1)_2$ in which the R^1 groups are independently selected, and defined herein before, and the phosphorus is attached through a carbon moiety (link, i.e. linker), which is attached to the rest of the molecule, e.g. drug or drug intermediate (R). The R^1 groups attached to the phosphonate esters in Scheme 1 may be changed using established chemical transformations. The interconversions may be carried out in the precursor compounds or the final products using the methods described below. The methods employed for a given phosphonate transformation depend on the nature of the substituent R^1 . The preparation and hydrolysis of phosphonate esters is described in *Organic Phosphorus Compounds*, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 9ff.

The conversion of a phosphonate diester **27.1** into the corresponding phosphonate monoester **27.2** (Scheme A, Reaction 1) can be accomplished by a number of methods. For example, the ester **27.1** in which R^1 is an arylalkyl group such as benzyl, can be converted into

the monoester compound 27.2 by reaction with a tertiary organic base such as diazabicyclooctane (DABCO) or quinuclidine, as described in *J. Org. Chem.*, 1995, 60:2946. The reaction is performed in an inert hydrocarbon solvent such as toluene or xylene, at about 110°C. The conversion of the diester 27.1 in which R¹ is an aryl group such as phenyl, or an alkenyl group such as allyl, into the monoester 27.2 can be effected by treatment of the ester 27.1 with a base such as aqueous sodium hydroxide in acetonitrile or lithium hydroxide in aqueous tetrahydrofuran. Phosphonate diesters 27.2 in which one of the groups R¹ is arylalkyl, such as benzyl, and the other is alkyl, can be converted into the monoesters 27.2 in which R¹ is alkyl, by hydrogenation, for example using a palladium on carbon catalyst. Phosphonate diesters in which both of the groups R¹ are alkenyl, such as allyl, can be converted into the monoester 27.2 in which R¹ is alkenyl, by treatment with chlorotris(triphenylphosphine)rhodium (Wilkinson's catalyst) in aqueous ethanol at reflux, optionally in the presence of diazabicyclooctane, for example by using the procedure described in *J. Org. Chem.*, 38:3224 1973 for the cleavage of allyl carboxylates.

The conversion of a phosphonate diester 27.1 or a phosphonate monoester 27.2 into the corresponding phosphonic acid 27.3 (Scheme A, Reactions 2 and 3) can be effected by reaction of the diester or the monoester with trimethylsilyl bromide, as described in *J. Chem. Soc., Chem. Comm.*, 739, 1979. The reaction is conducted in an inert solvent such as, for example, dichloromethane, optionally in the presence of a silylating agent such as bis(trimethylsilyl)trifluoroacetamide, at ambient temperature. A phosphonate monoester 27.2 in which R¹ is arylalkyl such as benzyl, can be converted into the corresponding phosphonic acid 27.3 by hydrogenation over a palladium catalyst, or by treatment with hydrogen chloride in an ethereal solvent such as dioxane. A phosphonate monoester 27.2 in which R¹ is alkenyl such as, for example, allyl, can be converted into the phosphonic acid 27.3 by reaction with Wilkinson's catalyst in an aqueous organic solvent, for example in 15% aqueous acetonitrile, or in aqueous ethanol, for example using the procedure described in *Helv. Chim. Acta.*, 68:618, 1985. Palladium catalyzed hydrogenolysis of phosphonate esters 27.1 in which R¹ is benzyl is described in *J. Org. Chem.*, 24:434, 1959. Platinum-catalyzed hydrogenolysis of phosphonate esters 27.1 in which R¹ is phenyl is described in *J. Amer. Chem. Soc.*, 78:2336, 1956.

The conversion of a phosphonate monoester 27.2 into a phosphonate diester 27.1 (Scheme A, Reaction 4) in which the newly introduced R¹ group is alkyl, arylalkyl, or haloalkyl such as chloroethyl, can be effected by a number of reactions in which the substrate 27.2 is reacted with a hydroxy compound R¹OH, in the presence of a coupling agent. Suitable

coupling agents are those employed for the preparation of carboxylate esters, and include a carbodiimide such as dicyclohexylcarbodiimide, in which case the reaction is preferably conducted in a basic organic solvent such as pyridine, or (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PYBOP, Sigma), in which case the reaction is performed in a polar solvent such as dimethylformamide, in the presence of a tertiary organic base such as diisopropylethylamine, or Aldrithiol-2 (Aldrich) in which case the reaction is conducted in a basic solvent such as pyridine, in the presence of a triaryl phosphine such as triphenylphosphine. Alternatively, the conversion of the phosphonate monoester **27.1** to the diester **27.1** can be effected by the use of the Mitsunobu reaction. The substrate is reacted with the hydroxy compound R^1OH , in the presence of diethyl azodicarboxylate and a triarylphosphine such as triphenyl phosphine. Alternatively, the phosphonate monoester **27.2** can be transformed into the phosphonate diester **27.1**, in which the introduced R^1 group is alkenyl or arylalkyl, by reaction of the monoester with the halide R^1Br , in which R^1 is as alkenyl or arylalkyl. The alkylation reaction is conducted in a polar organic solvent such as dimethylformamide or acetonitrile, in the presence of a base such as cesium carbonate. Alternatively, the phosphonate monoester can be transformed into the phosphonate diester in a two step procedure. In the first step, the phosphonate monoester **27.2** is transformed into the chloro analog $-P(O)(OR^1)Cl$ by reaction with thionyl chloride or oxalyl chloride and the like, as described in Organic Phosphorus Compounds, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 17, and the thus-obtained product $-P(O)(OR^1)Cl$ is then reacted with the hydroxy compound R^1OH , in the presence of a base such as triethylamine, to afford the phosphonate diester **27.1**.

A phosphonic acid $-P(O)(OH)_2$ can be transformed into a phosphonate monoester $-P(O)(OR^1)(OH)$ (Scheme A, Reaction 5) by means of the methods described above of for the preparation of the phosphonate diester $-P(O)(OR^1)_2$ **27.1**, except that only one molar proportion of the component R^1OH or R^1Br is employed.

A phosphonic acid $-P(O)(OH)_2$ **27.3** can be transformed into a phosphonate diester $-P(O)(OR^1)_2$ **27.1** (Scheme A, Reaction 6) by a coupling reaction with the hydroxy compound R^1OH , in the presence of a coupling agent such as Aldrithiol-2 (Aldrich) and triphenylphosphine. The reaction is conducted in a basic solvent such as pyridine. Alternatively, phosphonic acids **27.3** can be transformed into phosphonic esters **27.1** in which R^1 is aryl, such as phenyl, by means of a coupling reaction employing, for example, phenol and dicyclohexylcarbodiimide in pyridine at about 70°C. Alternatively, phosphonic acids **27.3** can be transformed into phosphonic esters **27.1** in which R^1 is alkenyl, by means of an alkylation

reaction. The phosphonic acid is reacted with the alkenyl bromide R^1Br in a polar organic solvent such as acetonitrile solution at reflux temperature, in the presence of a base such as cesium carbonate, to afford the phosphonic ester 27.1.

Phosphonate prodrugs of the present invention may also be prepared from the precursor free acid by Mitsunobu reactions (Mitsunobu, (1981) *Synthesis*, 1; Campbell, (1992) *J. Org. Chem.*, 52:6331), and other acid coupling reagents including, but not limited to, carbodiimides (Alexander, et al, (1994) *Collect. Czech. Chem. Commun.* 59:1853; Casara, et al, (1992) *Bioorg. Med. Chem. Lett.*, 2:145; Ohashi, et al, (1988) *Tetrahedron Lett.*, 29:1189), and benzotriazolyloxytris-(dimethylamino)phosphonium salts (Campagne, et al, (1993) *Tetrahedron Lett.*, 34:6743).

Preparation of carboalkoxy-substituted phosphonate bisamidates, monoamidates, diesters and monoesters.

A number of methods are available for the conversion of phosphonic acids into amidates and esters. In one group of methods, the phosphonic acid is either converted into an isolated activated intermediate such as a phosphoryl chloride, or the phosphonic acid is activated in situ for reaction with an amine or a hydroxy compound.

The conversion of phosphonic acids into phosphoryl chlorides is accomplished by reaction with thionyl chloride, for example as described in *J. Gen. Chem. USSR*, 1983, 53, 480, *Zh. Obschei Khim.*, 1958, 28, 1063, or *J. Org. Chem.*, 1994, 59, 6144, or by reaction with oxalyl chloride, as described in *J. Am. Chem. Soc.*, 1994, 116, 3251, or *J. Org. Chem.*, 1994, 59, 6144, or by reaction with phosphorus pentachloride, as described in *J. Org. Chem.*, 2001, 66, 329, or in *J. Med. Chem.*, 1995, 38, 1372. The resultant phosphoryl chlorides are then reacted with amines or hydroxy compounds in the presence of a base to afford the amidate or ester products.

Phosphonic acids are converted into activated imidazolyl derivatives by reaction with carbonyl diimidazole, as described in *J. Chem. Soc., Chem. Comm.*, 1991, 312, or *Nucleosides Nucleotides* 2000, 19, 1885. Activated sulfonyloxy derivatives are obtained by the reaction of phosphonic acids with trichloromethylsulfonyl chloride, as described in *J. Med. Chem.* 1995, 38, 4958, or with triisopropylbenzenesulfonyl chloride, as described in *Tet. Lett.*, 1996, 7857,

or Bioorg. Med. Chem. Lett., 1998, 8, 663. The activated sulfonyloxy derivatives are then reacted with amines or hydroxy compounds to afford amidates or esters.

Alternatively, the phosphonic acid and the amine or hydroxy reactant are combined in the presence of a diimide coupling agent. The preparation of phosphonic amidates and esters by means of coupling reactions in the presence of dicyclohexyl carbodiimide is described, for example, in J. Chem. Soc., Chem. Comm., 1991, 312, or J. Med. Chem., 1980, 23, 1299 or Coll. Czech. Chem. Comm., 1987, 52, 2792. The use of ethyl dimethylaminopropyl carbodiimide for activation and coupling of phosphonic acids is described in Tet. Lett., 2001, 42, 8841, or Nucleosides Nucleotides, 2000, 19, 1885.

A number of additional coupling reagents have been described for the preparation of amidates and esters from phosphonic acids. The agents include Aldrithiol-2, and PYBOP and BOP, as described in J. Org. Chem., 1995, 60, 5214, and J. Med. Chem., 1997, 40, 3842, mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (MSNT), as described in J. Med. Chem., 1996, 39, 4958, diphenylphosphoryl azide, as described in J. Org. Chem., 1984, 49, 1158, 1-(2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (TPSNT) as described in Bioorg. Med. Chem. Lett., 1998, 8, 1013, bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP), as described in Tet. Lett., 1996, 37, 3997, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane, as described in Nucleosides Nucleotides 1995, 14, 871, and diphenyl chlorophosphate, as described in J. Med. Chem., 1988, 31, 1305.

Phosphonic acids are converted into amidates and esters by means of the Mitsunobu reaction, in which the phosphonic acid and the amine or hydroxy reactant are combined in the presence of a triaryl phosphine and a dialkyl azodicarboxylate. The procedure is described in Org. Lett., 2001, 3, 643, or J. Med. Chem., 1997, 40, 3842.

Phosphonic esters are also obtained by the reaction between phosphonic acids and halo compounds, in the presence of a suitable base. The method is described, for example, in Anal. Chem., 1987, 59, 1056, or J. Chem. Soc. Perkin Trans., I, 1993, 19, 2303, or J. Med. Chem., 1995, 38, 1372, or Tet. Lett., 2002, 43, 1161.

Schemes 1 - 4 illustrate the conversion of phosphonate esters and phosphonic acids into carboalkoxy-substituted phosphorobisamidates (Scheme 1), phosphoroamidates (Scheme 2), phosphonate monoesters (Scheme 3) and phosphonate diesters, (Scheme 4).

Scheme 1 illustrates various methods for the conversion of phosphonate diesters 1.1 into phosphorobisamidates 1.5. The diester 1.1, prepared as described previously, is hydrolyzed, either to the monoester 1.2 or to the phosphonic acid 1.6. The methods employed for these transformations are described above. The monoester 1.2 is converted into the monoamidate 1.3 by reaction with an aminoester 1.9, in which the group R^2 is H or alkyl, the group R^4 is an alkylene moiety such as, for example, $CHCH_3$, $CHPr^1$, $CH(CH_2Ph)$, $CH_2CH(CH_3)$ and the like, or a group present in natural or modified aminoacids, and the group R^5 is alkyl. The reactants are combined in the presence of a coupling agent such as a carbodiimide, for example dicyclohexyl carbodiimide, as described in J. Am. Chem. Soc., 1957, 79, 3575, optionally in the presence of an activating agent such as hydroxybenztriazole, to yield the amidate product 1.3. The amidate-forming reaction is also effected in the presence of coupling agents such as BOP, as described in J. Org. Chem., 1995, 60, 5214, Aldrithiol, PYBOP and similar coupling agents used for the preparation of amides and esters. Alternatively, the reactants 1.2 and 1.9 are transformed into the monoamidate 1.3 by means of a Mitsunobu reaction. The preparation of amidates by means of the Mitsunobu reaction is described in J. Med. Chem., 1995, 38, 2742. Equimolar amounts of the reactants are combined in an inert solvent such as tetrahydrofuran in the presence of a triaryl phosphine and a dialkyl azodicarboxylate. The thus-obtained monoamidate ester 1.3 is then transformed into amidate phosphonic acid 1.4. The conditions used for the hydrolysis reaction depend on the nature of the R^1 group, as described previously. The phosphonic acid amidate 1.4 is then reacted with an aminoester 1.9, as described above, to yield the bisamidate product 1.5, in which the amino substituents are the same or different.

An example of this procedure is shown in Scheme 1, Example 1. In this procedure, a dibenzyl phosphonate 1.14 is reacted with diazabicyclooctane (DABCO) in toluene at reflux, as described in J. Org. Chem., 1995, 60, 2946, to afford the monobenzyl phosphonate 1.15. The product is then reacted with equimolar amounts of ethyl alaninate 1.16 and dicyclohexyl carbodiimide in pyridine, to yield the amidate product 1.17. The benzyl group is then removed, for example by hydrogenolysis over a palladium catalyst, to give the monoacid product 1.18. This compound is then reacted in a Mitsunobu reaction with ethyl leucinate 1.19, triphenyl phosphine and diethylazodicarboxylate, as described in J. Med. Chem., 1995, 38, 2742, to produce the bisamidate product 1.20.

Using the above procedures, but employing, in place of ethyl leucinate **1.19** or ethyl alaninate **1.16**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

Alternatively, the phosphonic acid **1.6** is converted into the bisamidate **1.5** by use of the coupling reactions described above. The reaction is performed in one step, in which case the nitrogen-related substituents present in the product **1.5** are the same, or in two steps, in which case the nitrogen-related substituents can be different.

An example of the method is shown in Scheme 1, Example 2. In this procedure, a phosphonic acid **1.6** is reacted in pyridine solution with excess ethyl phenylalaninate **1.21** and dicyclohexylcarbodiimide, for example as described in *J. Chem. Soc., Chem. Comm.*, 1991, 1063, to give the bisamidate product **1.22**.

Using the above procedures, but employing, in place of ethyl phenylalaninate, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

As a further alternative, the phosphonic acid **1.6** is converted into the mono or bis-activated derivative **1.7**, in which Lv is a leaving group such as chloro, imidazolyl, triisopropylbenzenesulfonyloxy etc. The conversion of phosphonic acids into chlorides **1.7** (Lv = Cl) is effected by reaction with thionyl chloride or oxalyl chloride and the like, as described in *Organic Phosphorus Compounds*, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 17. The conversion of phosphonic acids into monoimidazolides **1.7** (Lv = imidazolyl) is described in *J. Med. Chem.*, 2002, 45, 1284 and in *J. Chem. Soc. Chem. Comm.*, 1991, 312. Alternatively, the phosphonic acid is activated by reaction with triisopropylbenzenesulfonyl chloride, as described in *Nucleosides and Nucleotides*, 2000, 10, 1885. The activated product is then reacted with the aminoester **1.9**, in the presence of a base, to give the bisamidate **1.5**. The reaction is performed in one step, in which case the nitrogen substituents present in the product **1.5** are the same, or in two steps, via the intermediate **1.11**, in which case the nitrogen substituents can be different.

Examples of these methods are shown in Scheme 1, Examples 3 and 5. In the procedure illustrated in Scheme 1, Example 3, a phosphonic acid **1.6** is reacted with ten molar equivalents of thionyl chloride, as described in *Zh. Obshchei Khim.*, 1958, 28, 1063, to give the dichloro compound **1.23**. The product is then reacted at reflux temperature in a polar aprotic

solvent such as acetonitrile, and in the presence of a base such as triethylamine, with butyl serinate **1.24** to afford the bisamidate product **1.25**.

Using the above procedures, but employing, in place of butyl serinate **1.24**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

In the procedure illustrated in Scheme 1, Example 5, the phosphonic acid **1.6** is reacted, as described in J. Chem. Soc. Chem. Comm., 1991, 312, with carbonyl diimidazole to give the imidazolide **1.32**. The product is then reacted in acetonitrile solution at ambient temperature, with one molar equivalent of ethyl alaninate **1.33** to yield the monodisplacement product **1.34**. The latter compound is then reacted with carbonyl diimidazole to produce the activated intermediate **1.35**, and the product is then reacted, under the same conditions, with ethyl N-methylalaninate **1.33a** to give the bisamidate product **1.36**.

Using the above procedures, but employing, in place of ethyl alaninate **1.33** or ethyl N-methylalaninate **1.33a**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

The intermediate monoamidate **1.3** is also prepared from the monoester **1.2** by first converting the monoester into the activated derivative **1.8** in which Lv is a leaving group such as halo, imidazolyl etc, using the procedures described above. The product **1.8** is then reacted with an aminoester **1.9** in the presence of a base such as pyridine, to give an intermediate monoamidate product **1.3**. The latter compound is then converted, by removal of the R¹ group and coupling of the product with the aminoester **1.9**, as described above, into the bisamidate **1.5**.

An example of this procedure, in which the phosphonic acid is activated by conversion to the chloro derivative **1.26**, is shown in Scheme 1, Example 4. In this procedure, the phosphonic monobenzyl ester **1.15** is reacted, in dichloromethane, with thionyl chloride, as described in Tet. Let., 1994, 35, 4097, to afford the phosphoryl chloride **1.26**. The product is then reacted in acetonitrile solution at ambient temperature with one molar equivalent of ethyl 3-amino-2-methylpropionate **1.27** to yield the monoamidate product **1.28**. The latter compound is hydrogenated in ethyl acetate over a 5% palladium on carbon catalyst to produce the monoacid product **1.29**. The product is subjected to a Mitsunobu coupling procedure, with equimolar amounts of butyl alaninate **1.30**, triphenyl phosphine, diethylazodicarboxylate and triethylamine in tetrahydrofuran, to give the bisamidate product **1.31**.

Using the above procedures, but employing, in place of ethyl 3-amino-2-methylpropionate 1.27 or butyl alaninate 1.30, different aminoesters 1.9, the corresponding products 1.5 are obtained.

The activated phosphonic acid derivative 1.7 is also converted into the bisamidate 1.5 via the diamino compound 1.10. The conversion of activated phosphonic acid derivatives such as phosphoryl chlorides into the corresponding amino analogs 1.10, by reaction with ammonia, is described in Organic Phosphorus Compounds, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976. The diamino compound 1.10 is then reacted at elevated temperature with a haloester 1.12, in a polar organic solvent such as dimethylformamide, in the presence of a base such as dimethylaminopyridine or potassium carbonate, to yield the bisamidate 1.5. An example of this procedure is shown in Scheme 1, Example 6. In this method, a dichlorophosphonate 1.23 is reacted with ammonia to afford the diamide 1.37. The reaction is performed in aqueous, aqueous alcoholic or alcoholic solution, at reflux temperature. The resulting diamino compound is then reacted with two molar equivalents of ethyl 2-bromo-3-methylbutyrate 1.38, in a polar organic solvent such as N-methylpyrrolidinone at ca. 150°C, in the presence of a base such as potassium carbonate, and optionally in the presence of a catalytic amount of potassium iodide, to afford the bisamidate product 1.39.

Using the above procedures, but employing, in place of ethyl 2-bromo-3-methylbutyrate 1.38, different haloesters 1.12 the corresponding products 1.5 are obtained.

The procedures shown in Scheme 1 are also applicable to the preparation of bisamidates in which the aminoester moiety incorporates different functional groups. Scheme 1, Example 7 illustrates the preparation of bisamidates derived from tyrosine. In this procedure, the monoimidazolide 1.32 is reacted with propyl tyrosinate 1.40, as described in Example 5, to yield the monoamidate 1.41. The product is reacted with carbonyl diimidazole to give the imidazolide 1.42, and this material is reacted with a further molar equivalent of propyl tyrosinate to produce the bisamidate product 1.43.

Using the above procedures, but employing, in place of propyl tyrosinate 1.40, different aminoesters 1.9, the corresponding products 1.5 are obtained. The aminoesters employed in

the two stages of the above procedure can be the same or different, so that bisamidates with the same or different amino substituents are prepared.

Scheme 2 illustrates methods for the preparation of phosphonate monoamidates.

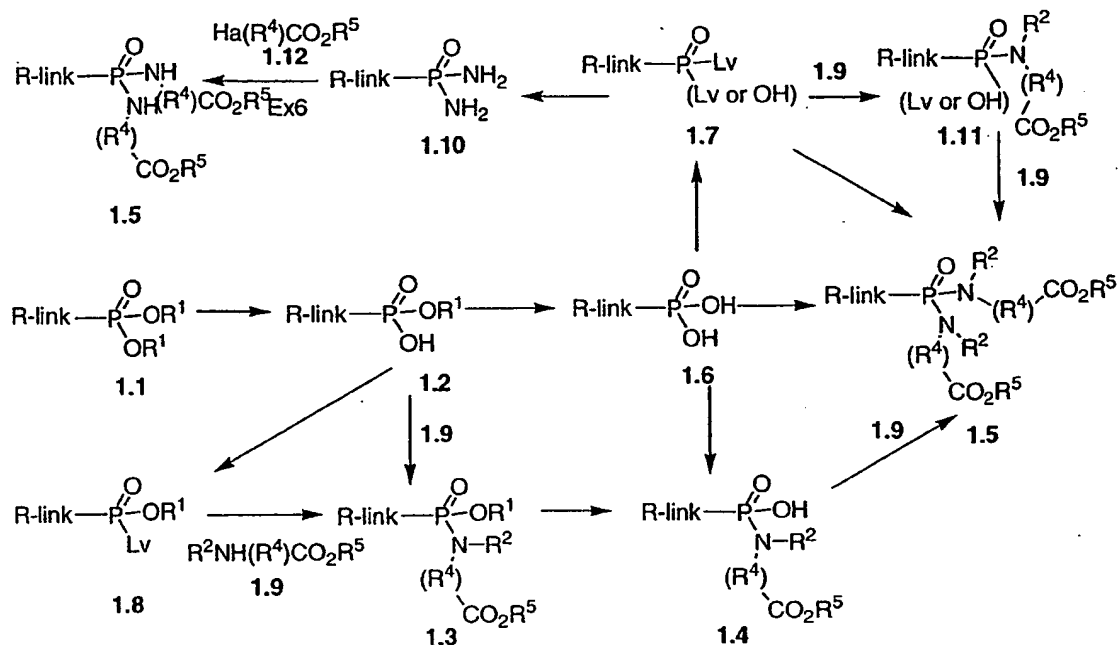
In one procedure, a phosphonate monoester **1.1** is converted, as described in Scheme 1, into the activated derivative **1.8**. This compound is then reacted, as described above, with an aminoester **1.9**, in the presence of a base, to afford the monoamidate product **2.1**.

The procedure is illustrated in Scheme 2, Example 1. In this method, a monophenyl phosphonate **2.7** is reacted with, for example, thionyl chloride, as described in J. Gen. Chem. USSR., 1983, 32, 367, to give the chloro product **2.8**. The product is then reacted, as described in Scheme 1, with ethyl alaninate **2.9**, to yield the amidate **2.10**.

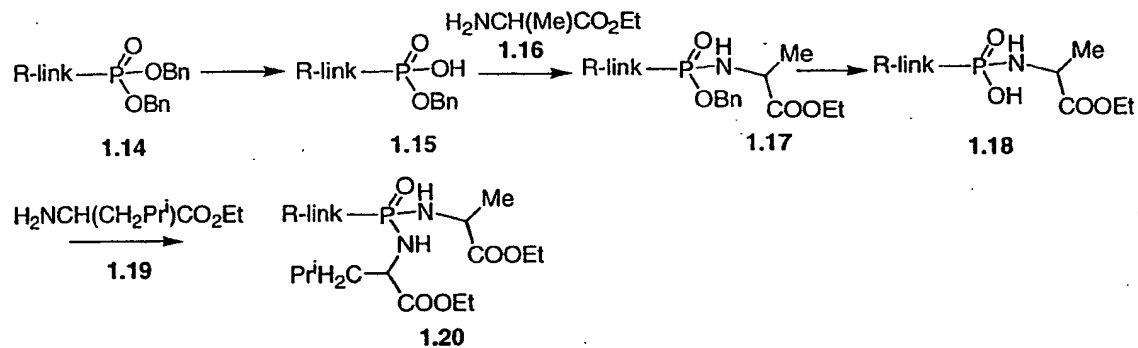
Using the above procedures, but employing, in place of ethyl alaninate **2.9**, different aminoesters **1.9**, the corresponding products **2.1** are obtained.

Alternatively, the phosphonate monoester **1.1** is coupled, as described in Scheme 1, with an aminoester **1.9** to produce the amidate **2.1**. If necessary, the R¹ substituent is then altered, by initial cleavage to afford the phosphonic acid **2.2**. The procedures for this transformation depend on the nature of the R¹ group, and are described above. The phosphonic acid is then transformed into the ester amidate product **2.3**, by reaction with the hydroxy compound R³OH, in which the group R³ is aryl, heteroaryl, alkyl, cycloalkyl, haloalkyl etc, using the same coupling procedures (carbodiimide, Aldrithiol-2, PYBOP, Mitsunobu reaction etc) described in Scheme 1 for the coupling of amines and phosphonic acids.

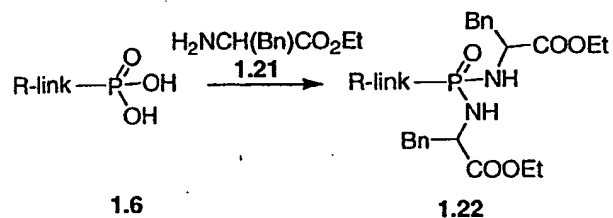
Scheme 1



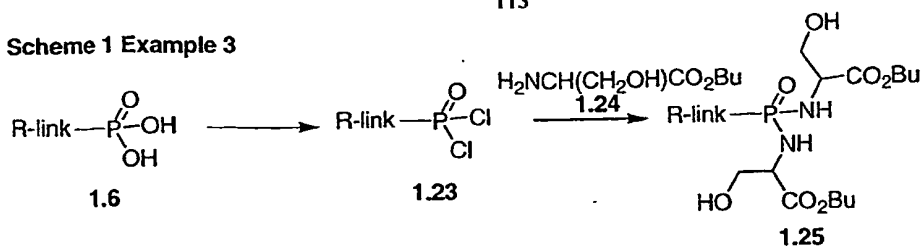
Scheme 1 Example 1



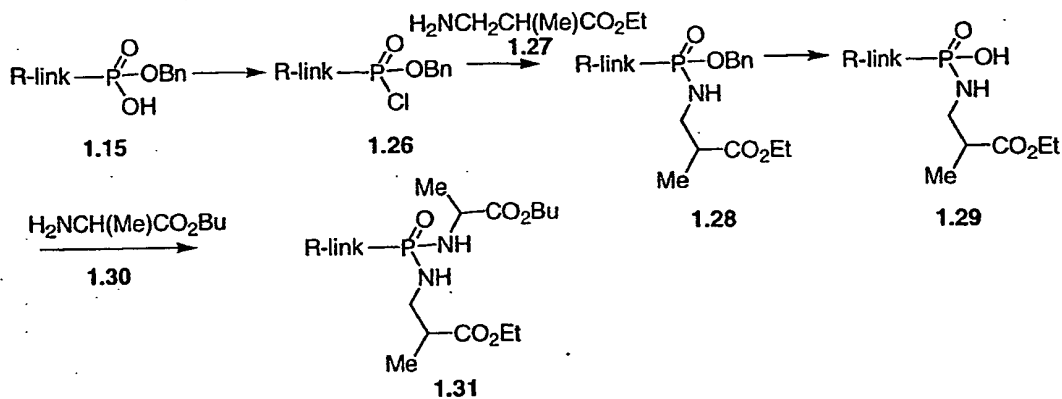
Scheme 1 Example 2



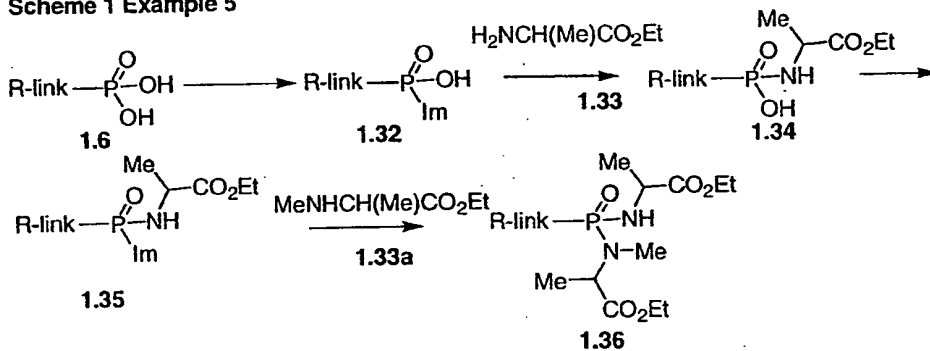
Scheme 1 Example 3



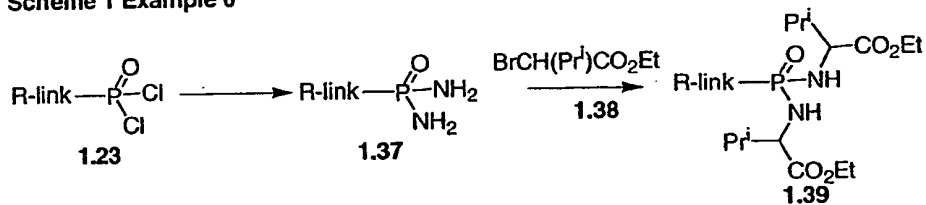
Scheme 1 Example 4



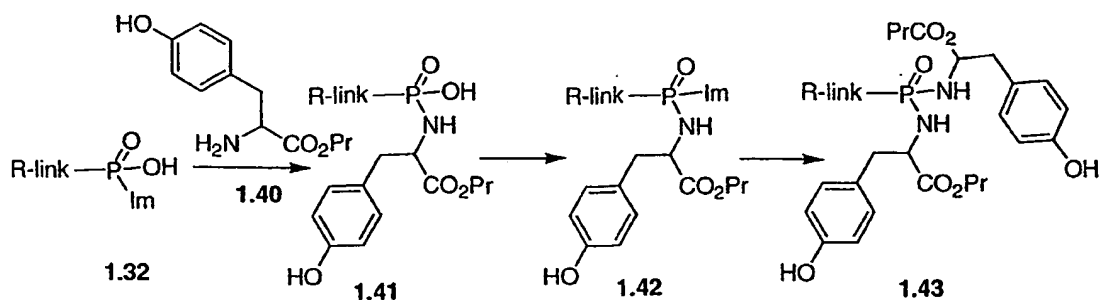
Scheme 1 Example 5



Scheme 1 Example 6



Scheme 1 Example 7



Examples of this method are shown in Scheme 2, Examples and 2 and 3. In the sequence shown in Example 2, a monobenzyl phosphonate 2.11 is transformed by reaction with ethyl alaninate, using one of the methods described above, into the monoamidate 2.12. The benzyl group is then removed by catalytic hydrogenation in ethyl acetate solution over a 5% palladium on carbon catalyst, to afford the phosphonic acid amidate 2.13. The product is then reacted in dichloromethane solution at ambient temperature with equimolar amounts of 1-(dimethylaminopropyl)-3-ethylcarbodiimide and trifluoroethanol 2.14, for example as described in Tet. Lett., 2001, 42, 8841, to yield the amidate ester 2.15.

In the sequence shown in Scheme 2, Example 3, the monoamidate 2.13 is coupled, in tetrahydrofuran solution at ambient temperature, with equimolar amounts of dicyclohexyl carbodiimide and 4-hydroxy-N-methylpiperidine 2.16, to produce the amidate ester product 2.17.

Using the above procedures, but employing, in place of the ethyl alaninate product 2.12 different monoacids 2.2, and in place of trifluoroethanol 2.14 or 4-hydroxy-N-methylpiperidine 2.16, different hydroxy compounds R³OH, the corresponding products 2.3 are obtained.

Alternatively, the activated phosphonate ester 1.8 is reacted with ammonia to yield the amidate 2.4. The product is then reacted, as described in Scheme 1, with a haloester 2.5, in the presence of a base, to produce the amidate product 2.6. If appropriate, the nature of the R¹ group is changed, using the procedures described above, to give the product 2.3. The method is illustrated in Scheme 2, Example 4. In this sequence, the monophenyl phosphoryl chloride 2.18 is reacted, as described in Scheme 1, with ammonia, to yield the amino product 2.19.

This material is then reacted in N-methylpyrrolidinone solution at 170°C with butyl 2-bromo-3-phenylpropionate **2.20** and potassium carbonate, to afford the amidate product **2.21**.

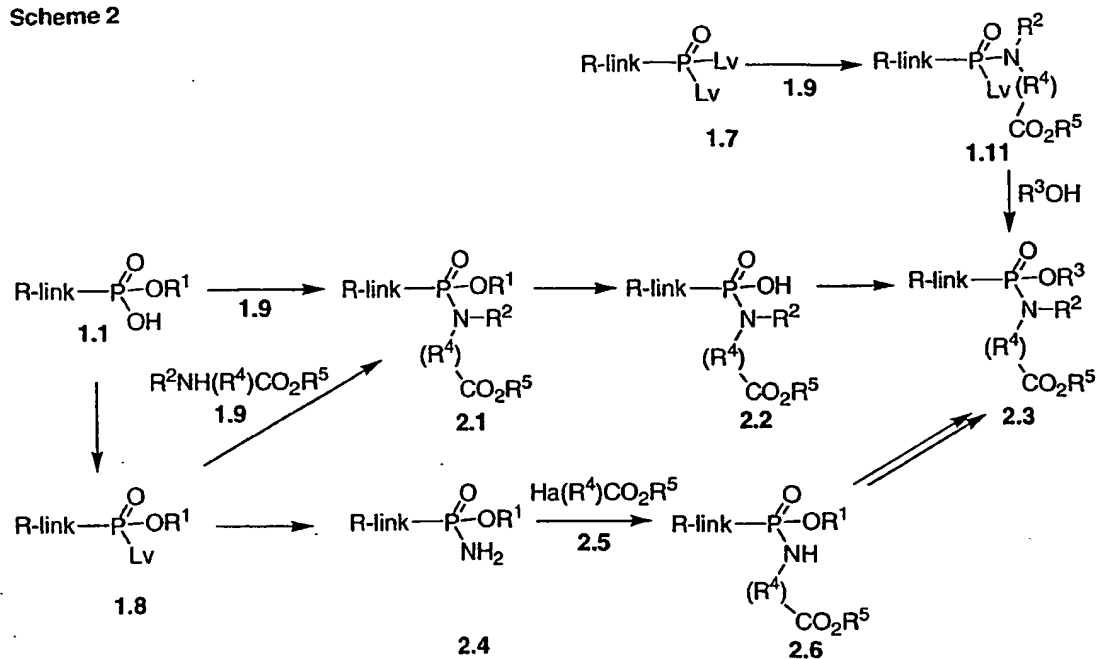
Using these procedures, but employing, in place of butyl 2-bromo-3-phenylpropionate **2.20**, different haloesters **2.5**, the corresponding products **2.6** are obtained.

The monoamidate products **2.3** are also prepared from the doubly activated phosphonate derivatives **1.7**. In this procedure, examples of which are described in Synlett., 1998, 1, 73, the intermediate **1.7** is reacted with a limited amount of the aminoester **1.9** to give the mono-displacement product **1.11**. The latter compound is then reacted with the hydroxy compound R^3OH in a polar organic solvent such as dimethylformamide, in the presence of a base such as diisopropylethylamine, to yield the monoamidate ester **2.3**.

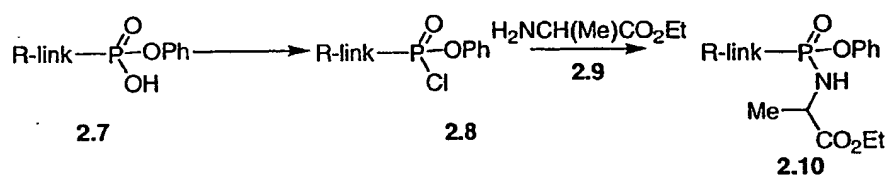
The method is illustrated in Scheme 2, Example 5. In this method, the phosphoryl dichloride **2.22** is reacted in dichloromethane solution with one molar equivalent of ethyl N-methyl tyrosinate **2.23** and dimethylaminopyridine, to generate the monoamidate **2.24**. The product is then reacted with phenol **2.25** in dimethylformamide containing potassium carbonate, to yield the ester amidate product **2.26**.

Using these procedures, but employing, in place of ethyl N-methyl tyrosinate **2.23** or phenol **2.25**, the aminoesters **1.9** and/or the hydroxy compounds R^3OH , the corresponding products **2.3** are obtained.

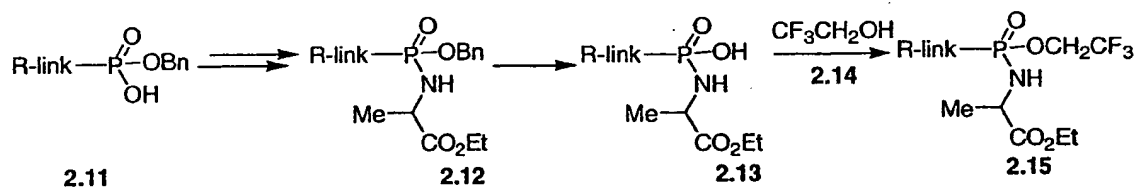
Scheme 2



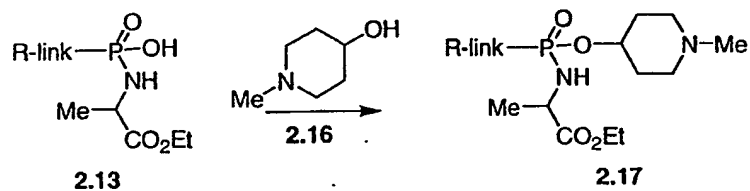
Scheme 2 Example 1



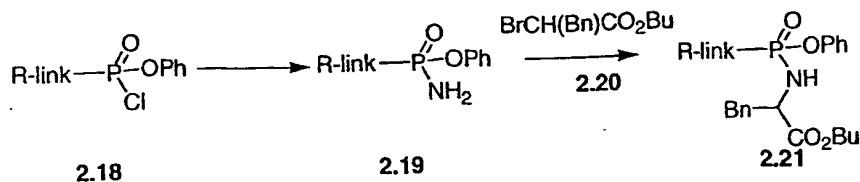
Scheme 2 Example 2



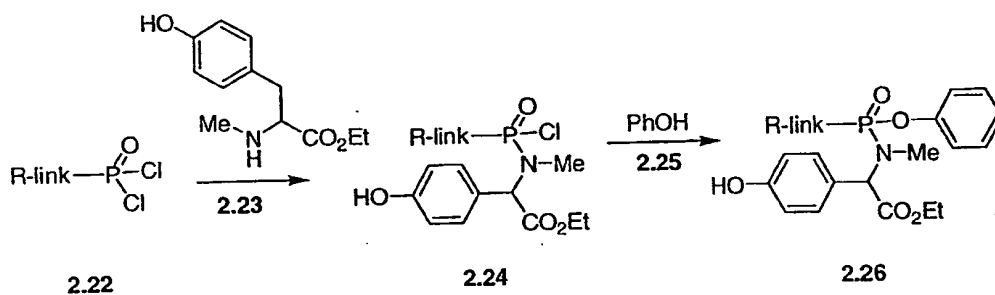
Scheme 2 Example 3



Scheme 2 Example 4



Scheme 2 Example 5



Scheme 3 illustrates methods for the preparation of carboalkoxy-substituted phosphonate diesters in which one of the ester groups incorporates a carboalkoxy substituent.

In one procedure, a phosphonate monoester 1.1, prepared as described above, is coupled, using one of the methods described above, with a hydroxyester 3.1, in which the groups R^4 and R^5 are as described in Scheme 1. For example, equimolar amounts of the reactants are coupled in the presence of a carbodiimide such as dicyclohexyl carbodiimide, as described in Aust. J. Chem., 1963, 609, optionally in the presence of dimethylaminopyridine, as described in Tet., 1999, 55, 12997. The reaction is conducted in an inert solvent at ambient temperature.

The procedure is illustrated in Scheme 3, Example 1. In this method, a monophenyl phosphonate 3.9 is coupled, in dichloromethane solution in the presence of dicyclohexyl carbodiimide, with ethyl 3-hydroxy-2-methylpropionate 3.10 to yield the phosphonate mixed diester 3.11.

Using this procedure, but employing, in place of ethyl 3-hydroxy-2-methylpropionate 3.10, different hydroxyesters 3.1, the corresponding products 3.2 are obtained.

The conversion of a phosphonate monoester **1.1** into a mixed diester **3.2** is also accomplished by means of a Mitsunobu coupling reaction with the hydroxyester **3.1**, as described in Org. Lett., 2001, 643. In this method, the reactants **1.1** and **3.1** are combined in a polar solvent such as tetrahydrofuran, in the presence of a triarylphosphine and a dialkyl azodicarboxylate, to give the mixed diester **3.2**. The R¹ substituent is varied by cleavage, using the methods described previously, to afford the monoacid product **3.3**. The product is then coupled, for example using methods described above, with the hydroxy compound R³OH, to give the diester product **3.4**.

The procedure is illustrated in Scheme 3, Example 2. In this method, a monoallyl phosphonate **3.12** is coupled in tetrahydrofuran solution, in the presence of triphenylphosphine and diethylazodicarboxylate, with ethyl lactate **3.13** to give the mixed diester **3.14**. The product is reacted with tris(triphenylphosphine) rhodium chloride (Wilkinson catalyst) in acetonitrile, as described previously, to remove the allyl group and produce the monoacid product **3.15**. The latter compound is then coupled, in pyridine solution at ambient temperature, in the presence of dicyclohexyl carbodiimide, with one molar equivalent of 3-hydroxypyridine **3.16** to yield the mixed diester **3.17**.

Using the above procedures, but employing, in place of the ethyl lactate **3.13** or 3-hydroxypyridine, a different hydroxyester **3.1** and/or a different hydroxy compound R³OH, the corresponding products **3.4** are obtained.

The mixed diesters **3.2** are also obtained from the monoesters **1.1** via the intermediacy of the activated monoesters **3.5**. In this procedure, the monoester **1.1** is converted into the activated compound **3.5** by reaction with, for example, phosphorus pentachloride, as described in J. Org. Chem., 2001, 66, 329, or with thionyl chloride or oxalyl chloride (Lv = Cl), or with triisopropylbenzenesulfonyl chloride in pyridine, as described in Nucleosides and Nucleotides, 2000, 19, 1885, or with carbonyl diimidazole, as described in J. Med. Chem., 2002, 45, 1284. The resultant activated monoester is then reacted with the hydroxyester **3.1**, as described above, to yield the mixed diester **3.2**.

The procedure is illustrated in Scheme 3, Example 3. In this sequence, a monophenyl phosphonate **3.9** is reacted, in acetonitrile solution at 70°C, with ten equivalents of thionyl chloride, so as to produce the phosphoryl chloride **3.19**. The product is then reacted with

ethyl 4-carbamoyl-2-hydroxybutyrate **3.20** in dichloromethane containing triethylamine, to give the mixed diester **3.21**.

Using the above procedures, but employing, in place of ethyl 4-carbamoyl-2-hydroxybutyrate **3.20**, different hydroxyesters **3.1**, the corresponding products **3.2** are obtained.

The mixed phosphonate diesters are also obtained by an alternative route for incorporation of the R^3O group into intermediates **3.3** in which the hydroxyester moiety is already incorporated. In this procedure, the monoacid intermediate **3.3** is converted into the activated derivative **3.6** in which Lv is a leaving group such as chloro, imidazole, and the like, as previously described. The activated intermediate is then reacted with the hydroxy compound R^3OH , in the presence of a base, to yield the mixed diester product **3.4**.

The method is illustrated in Scheme 3, Example 4. In this sequence, the phosphonate monoacid **3.22** is reacted with trichloromethanesulfonyl chloride in tetrahydrofuran containing collidine, as described in J. Med. Chem., 1995, 38, 4648, to produce the trichloromethanesulfonyloxy product **3.23**. This compound is reacted with 3-(morpholinomethyl)phenol **3.24** in dichloromethane containing triethylamine, to yield the mixed diester product **3.25**.

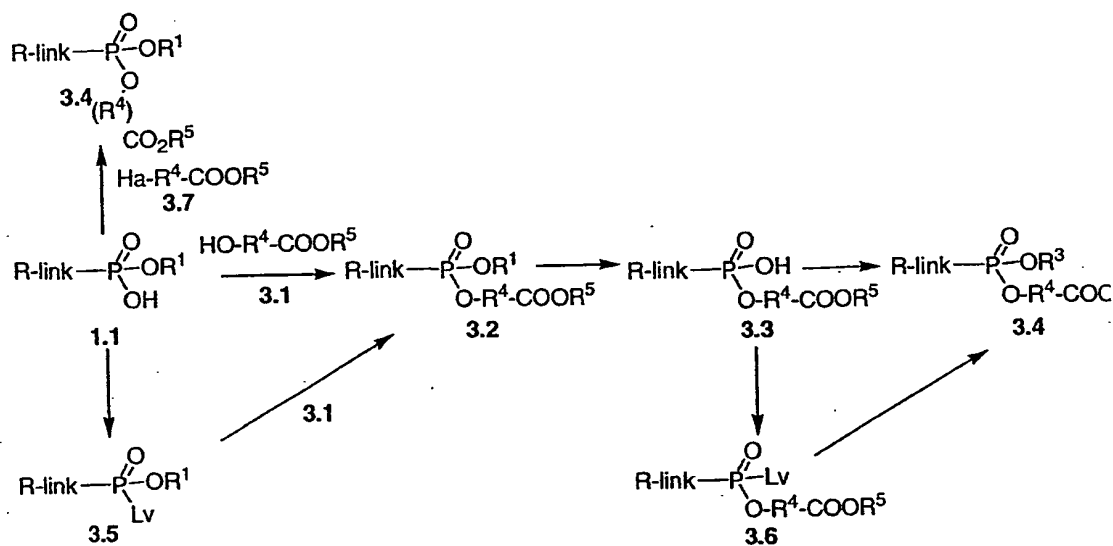
Using the above procedures, but employing, in place of with 3-(morpholinomethyl)phenol **3.24**, different carbinols R^3OH , the corresponding products **3.4** are obtained.

The phosphonate esters **3.4** are also obtained by means of alkylation reactions performed on the monoesters **1.1**. The reaction between the monoacid **1.1** and the haloester **3.7** is performed in a polar solvent in the presence of a base such as diisopropylethylamine, as described in Anal. Chem., 1987, 59, 1056, or triethylamine, as described in J. Med. Chem., 1995, 38, 1372, or in a non-polar solvent such as benzene, in the presence of 18-crown-6, as described in Syn. Comm., 1995, 25, 3565.

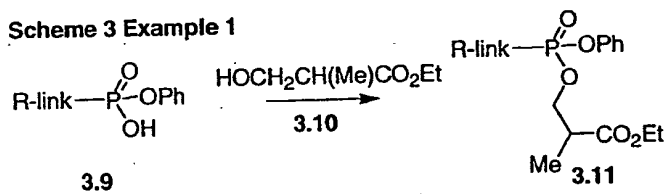
The method is illustrated in Scheme 3, Example 5. In this procedure, the monoacid **3.26** is reacted with ethyl 2-bromo-3-phenylpropionate **3.27** and diisopropylethylamine in dimethylformamide at 80°C to afford the mixed diester product **3.28**.

Using the above procedure, but employing, in place of ethyl 2-bromo-3-phenylpropionate 3.27, different haloesters 3.7, the corresponding products 3.4 are obtained.

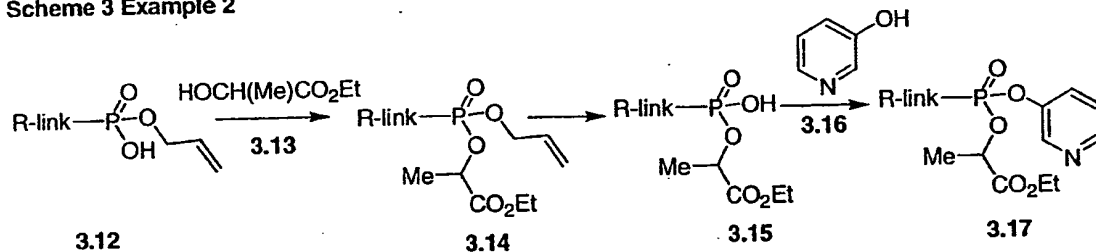
Scheme 3



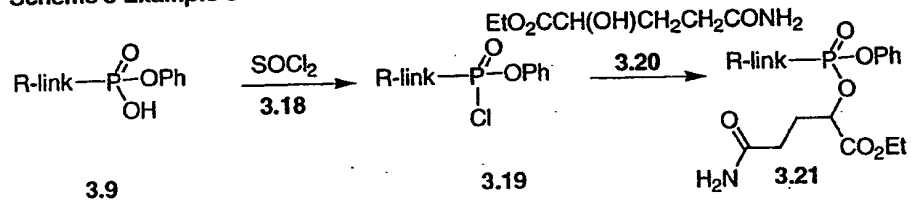
Scheme 3 Example 1



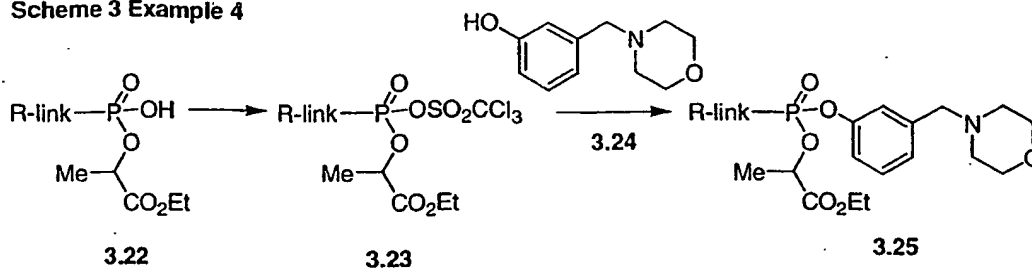
Scheme 3 Example 2



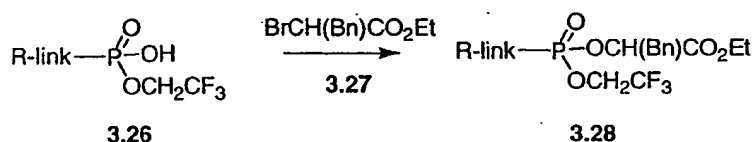
Scheme 3 Example 3



Scheme 3 Example 4



Scheme 3 Example 5



Scheme 4 illustrates methods for the preparation of phosphonate diesters in which both the ester substituents incorporate carboalkoxy groups.

The compounds are prepared directly or indirectly from the phosphonic acids 1.6. In one alternative, the phosphonic acid is coupled with the hydroxyester 4.2, using the conditions described previously in Schemes 1 - 3, such as coupling reactions using dicyclohexyl carbodiimide or similar reagents, or under the conditions of the Mitsunobu reaction, to afford the diester product 4.3 in which the ester substituents are identical.

This method is illustrated in Scheme 4, Example 1. In this procedure, the phosphonic acid 1.6 is reacted with three molar equivalents of butyl lactate 4.5 in the presence of Aldrithiol-2 and triphenyl phosphine in pyridine at ca. 70°C, to afford the diester 4.6.

Using the above procedure, but employing, in place of butyl lactate 4.5, different hydroxyesters 4.2, the corresponding products 4.3 are obtained.

Alternatively, the diesters 4.3 are obtained by alkylation of the phosphonic acid 1.6 with a haloester 4.1. The alkylation reaction is performed as described in Scheme 3 for the preparation of the esters 3.4.

This method is illustrated in Scheme 4, Example 2. In this procedure, the phosphonic acid **1.6** is reacted with excess ethyl 3-bromo-2-methylpropionate **4.7** and diisopropylethylamine in dimethylformamide at ca. 80°C, as described in Anal. Chem., 1987, 59, 1056, to produce the diester **4.8**.

Using the above procedure, but employing, in place of ethyl 3-bromo-2-methylpropionate **4.7**, different haloesters **4.1**, the corresponding products **4.3** are obtained.

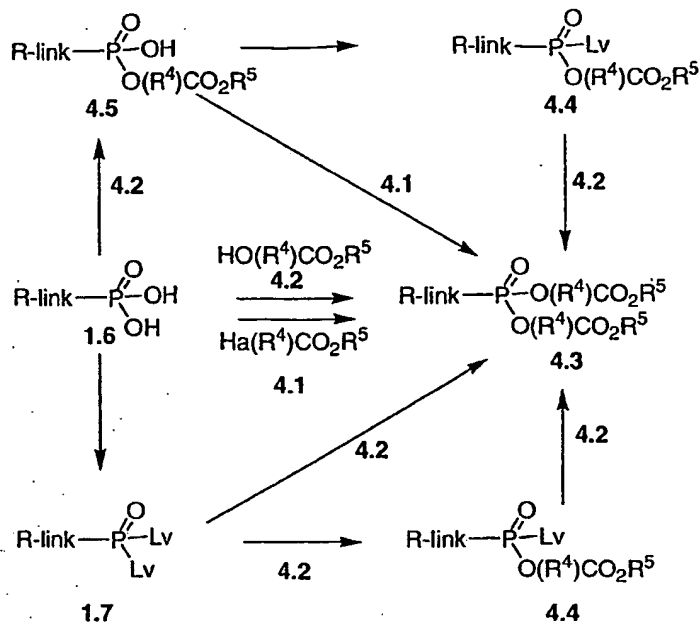
The diesters **4.3** are also obtained by displacement reactions of activated derivatives **1.7** of the phosphonic acid with the hydroxyesters **4.2**. The displacement reaction is performed in a polar solvent in the presence of a suitable base, as described in Scheme 3. The displacement reaction is performed in the presence of an excess of the hydroxyester, to afford the diester product **4.3** in which the ester substituents are identical, or sequentially with limited amounts of different hydroxyesters, to prepare diesters **4.3** in which the ester substituents are different. The methods are illustrated in Scheme 4, Examples 3 and 4. As shown in Example 3, the phosphoryl dichloride **2.22** is reacted with three molar equivalents of ethyl 3-hydroxy-2-(hydroxymethyl)propionate **4.9** in tetrahydrofuran containing potassium carbonate, to obtain the diester product **4.10**.

Using the above procedure, but employing, in place of ethyl 3-hydroxy-2-(hydroxymethyl)propionate **4.9**, different hydroxyesters **4.2**, the corresponding products **4.3** are obtained.

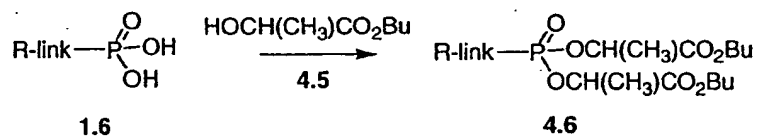
Scheme 4, Example 4 depicts the displacement reaction between equimolar amounts of the phosphoryl dichloride **2.22** and ethyl 2-methyl-3-hydroxypropionate **4.11**, to yield the monoester product **4.12**. The reaction is conducted in acetonitrile at 70°C in the presence of diisopropylethylamine. The product **4.12** is then reacted, under the same conditions, with one molar equivalent of ethyl lactate **4.13**, to give the diester product **4.14**.

Using the above procedures, but employing, in place of ethyl 2-methyl-3-hydroxypropionate **4.11** and ethyl lactate **4.13**, sequential reactions with different hydroxyesters **4.2**, the corresponding products **4.3** are obtained.

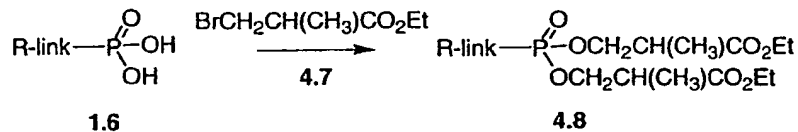
Scheme 4



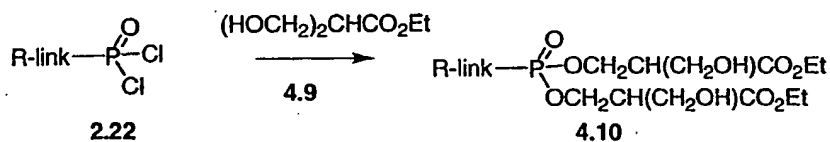
Scheme 4 Example 1



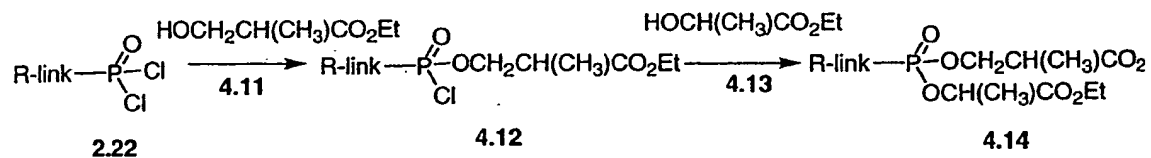
Scheme 4 Example 2



Scheme 4 Example 3



Scheme 4 Example 4



Aryl halides undergo Ni^{+2} catalyzed reaction with phosphite derivatives to give aryl phosphonate containing compounds (Balthazar, et al (1980) *J. Org. Chem.* 45:5425). Phosphonates may also be prepared from the chlorophosphonate in the presence of a palladium catalyst using aromatic triflates (Petrakis, et al, (1987) *J. Am. Chem. Soc.* 109:2831; Lu, et al, (1987) *Synthesis*, 726). In another method, aryl phosphonate esters are prepared from aryl phosphates under anionic rearrangement conditions (Melvin (1981) *Tetrahedron Lett.* 22:3375; Casteel, et al, (1991) *Synthesis*, 691). N-Alkoxy aryl salts with alkali metal derivatives of cyclic alkyl phosphonate provide general synthesis for heteroaryl-2-phosphonate linkers (Redmore (1970) *J. Org. Chem.* 35:4114). These above mentioned methods can also be extended to compounds where the W^5 group is a heterocycle. Cyclic-1,3-propanyl prodrugs of phosphonates are also synthesized from phosphonic diacids and substituted propane-1,3-diols using a coupling reagent such as 1,3-dicyclohexylcarbodiimide (DCC) in presence of a base (e.g., pyridine). Other carbodiimide based coupling agents like 1,3-disopropylcarbodiimide or water soluble reagent, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) can also be utilized for the synthesis of cyclic phosphonate prodrugs.

The carbamoyl group may be formed by reaction of a hydroxy group according to the methods known in the art, including the teachings of Ellis, US 2002/0103378 A1 and Hajima, US Patent No. 6018049.

Generally, the reaction conditions such as temperature, reaction time, solvents, work-up procedures, and the like, will be those common in the art for the particular reaction to be performed. The cited reference material, together with material cited therein, contains detailed descriptions of such conditions. Typically the temperatures will be -100°C to 200°C , solvents will be aprotic or protic, and reaction times will be 10 seconds to 10 days. Work-up typically consists of quenching any unreacted reagents followed by partition between a water/organic layer system (extraction) and separating the layer containing the product.

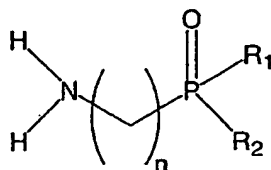
Oxidation and reduction reactions are typically carried out at temperatures near room temperature (about 20°C), although for metal hydride reductions frequently the temperature is reduced to 0°C to -100°C , solvents are typically aprotic for reductions and may be either protic or aprotic for oxidations. Reaction times are adjusted to achieve desired conversions.

Condensation reactions are typically carried out at temperatures near room temperature, although for non-equilibrating, kinetically controlled condensations reduced temperatures (0°C to -100°C) are also common. Solvents can be either protic (common in equilibrating reactions) or aprotic (common in kinetically controlled reactions).

Standard synthetic techniques such as azeotropic removal of reaction by-products and use of anhydrous reaction conditions (e.g. inert gas environments) are common in the art and will be applied when applicable.

General synthetic routes to substituted imidazoles are well established. See Ogata M (1988) *Annals of the New York Academy of Sciences* 544:12-31; Takahashi et al (1985) *Heterocycles* 23:6, 1483-1492; Ogata et al (1980) *CHEM IND LONDON* 2:5-86; Yanagisawa et al US Patent No. 5646171; Rachwal et al US 2002/0115693 A1; Carlson et al US Patent Nos. 3790593; 3761491 and 3773781; Aono et al US Patent No. 6054591; Hajima et al US Patent No. 6057448; Sugimoto et al EP 00552060 and US Patent No. 5326780.

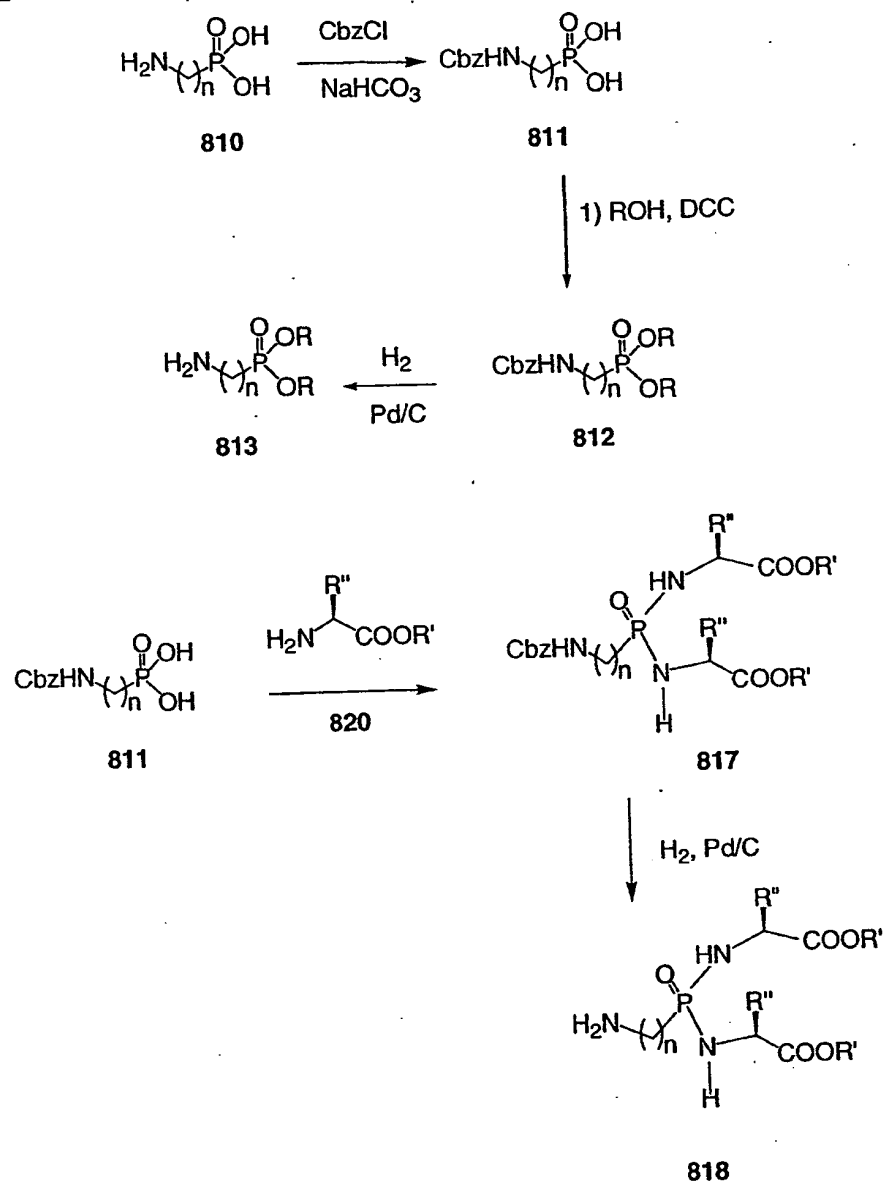
Amino alkyl phosphonate compounds **809**:

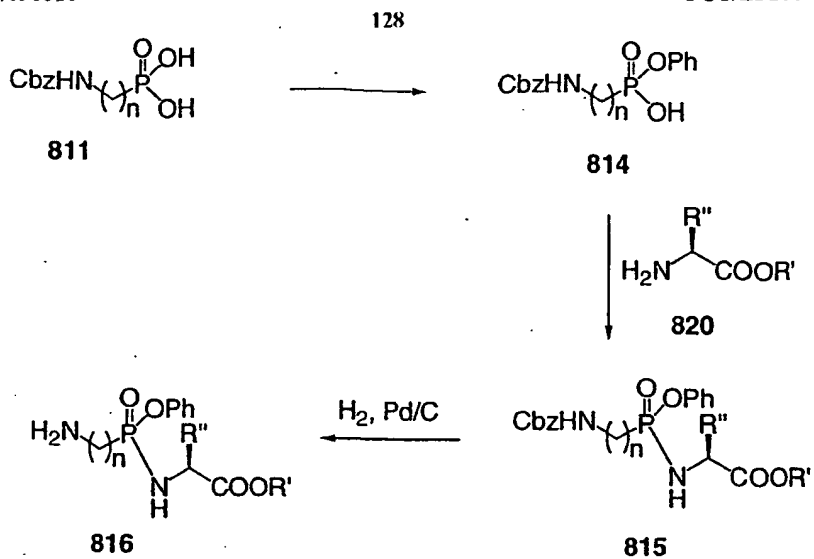


809

are a generic representative of compounds **811**, **813**, **814**, **816** and **818** (Scheme 2). The alkylene chain may be any length from 1 to 18 methylene groups ($n = 1-18$). Commercial amino phosphonic acid **810** was protected as carbamate **811**. The phosphonic acid **811** was converted to phosphonate **812** upon treatment with ROH in the presence of DCC or other conventional coupling reagents. Coupling of phosphonic acid **811** with esters of amino acid **820** provided bisamidate **817**. Conversion of acid **811** to bisphenyl phosphonate followed by hydrolysis gave mono-phosphonic acid **814** (Cbz = C₆H₅CH₂C(O)-), which was then transformed to mono-phosphonic amidate **815**. Carbamates **813**, **816** and **818** were converted to their corresponding amines upon hydrogenation. Compounds **811**, **813**, **814**, **816** and **818** are useful intermediates to form the phosphonate compounds of the invention.

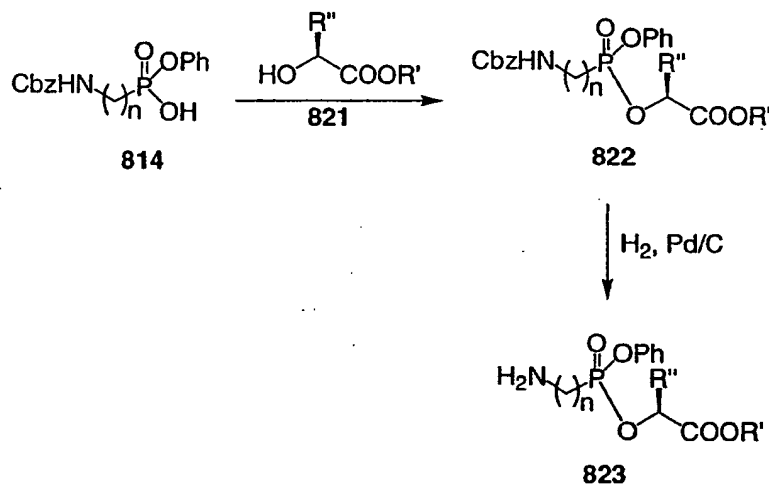
Scheme 2





Following the similar procedures, replacement of amino acid esters 820 with lactates 821 (Scheme 3) provides mono-phosphonic lactates 823. Lactates 823 are useful intermediates to form the phosphonate compounds of the invention.

Scheme 3



Examples General Section

The following Examples refer to the Schemes. Some Examples have been performed multiple times. In repeated Examples, reaction conditions such as time, temperature, concentration and the like, and yields were within normal experimental ranges. In repeated Examples where significant modifications were made, these have been noted where the results varied significantly from those described. In Examples where different starting materials were used, these are noted. When the repeated Examples refer to a "corresponding" analog of a compound, such as a "corresponding ethyl ester", this intends that an otherwise present group, in this case typically a methyl ester, is taken to be the same group modified as indicated.

Example 1

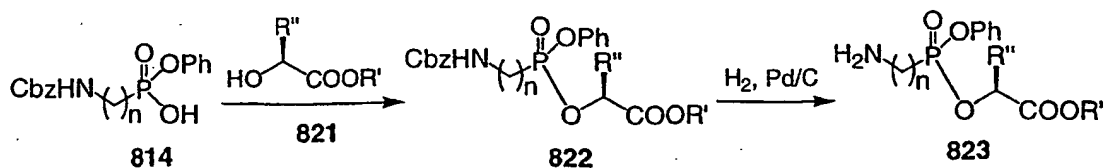
To a solution of 2-aminoethylphosphonic acid (**810** where $n=2$, 1.26 g, 10.1 mmol) in 2N NaOH (10.1 mL, 20.2 mmol) was added benzyl chloroformate (1.7 mL, 12.1 mmol). See Scheme 5. After the reaction mixture was stirred for 2 d at room temperature, the mixture was partitioned between Et₂O and water. The aqueous phase was acidified with 6N HCl until pH = 2. The resulting colorless solid was dissolved in MeOH (75 mL) and treated with Dowex 50WX8-200 (7 g). After the mixture was stirred for 30 minutes, it was filtered and evaporated under reduced pressure to give carbamate **28** (2.37 g, 91%) as a colorless solid.

To a solution of carbamate **28** (2.35 g, 9.1 mmol) in pyridine (40 mL) was added phenol (8.53 g, 90.6 mmol) and 1,3-dicyclohexylcarbodiimide (7.47 g, 36.2 mmol). After the reaction mixture was warmed to 70°C and stirred for 5 h, the mixture was diluted with CH₃CN and filtered. The filtrate was concentrated under reduced pressure and diluted with EtOAc. The organic phase was washed with sat. NH₄Cl, sat. NaHCO₃, and brine, then dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel twice (eluting 40-60% EtOAc/hexane) to give phosphonate **29** (2.13 g, 57%) as a colorless solid.

To a solution of phosphonate **29** (262 mg, 0.637 mmol) in iPrOH (5 mL) was added TFA (0.05 mL, 0.637 mmol) and 10% Pd/C (26 mg). After the reaction mixture was stirred under H₂ atmosphere (balloon) for 1 h, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give amine **30** (249 mg, 100%) as a colorless oil (Scheme 5).

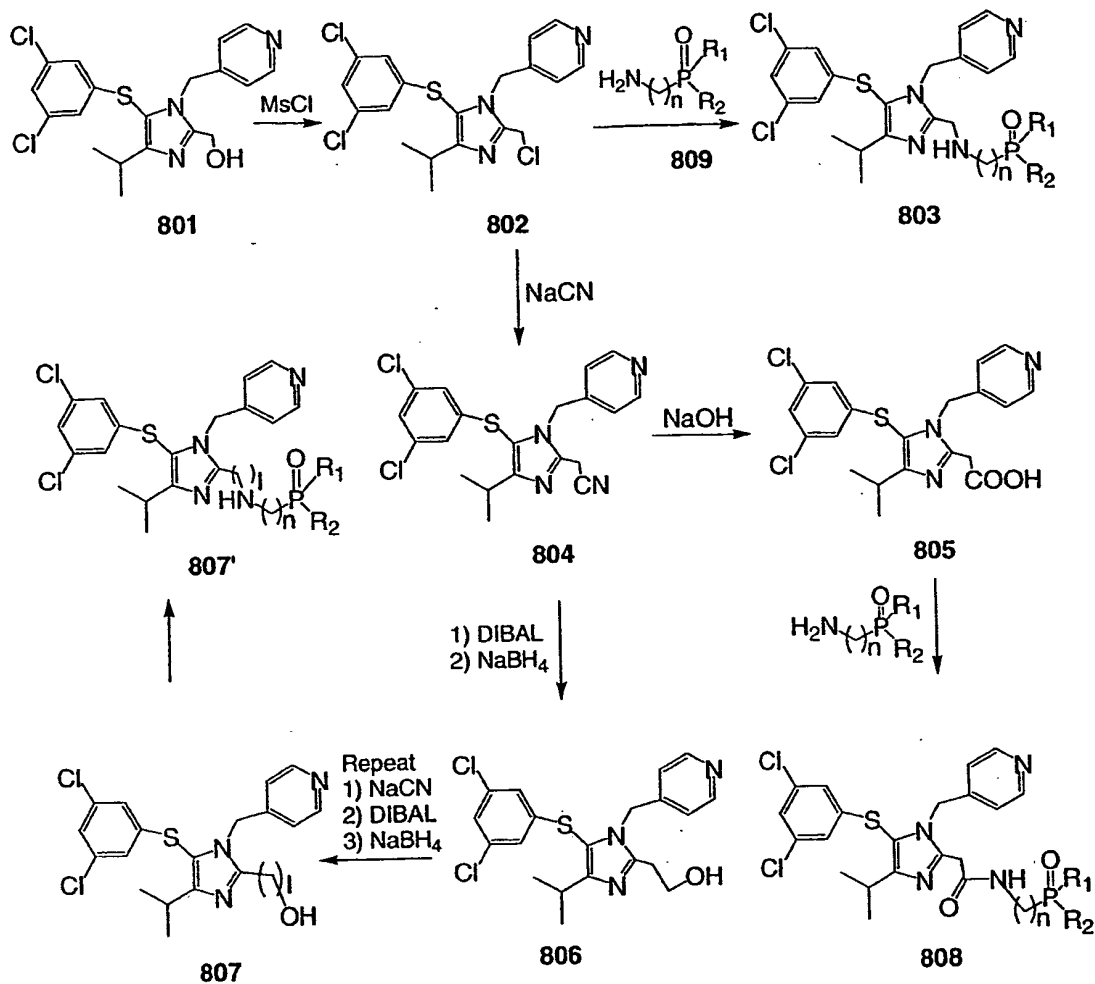
Following the similar procedures, replacement of amino acid esters with lactates (Scheme 6) provided mono-phosphonic lactates, e.g. **823**.

Scheme 6



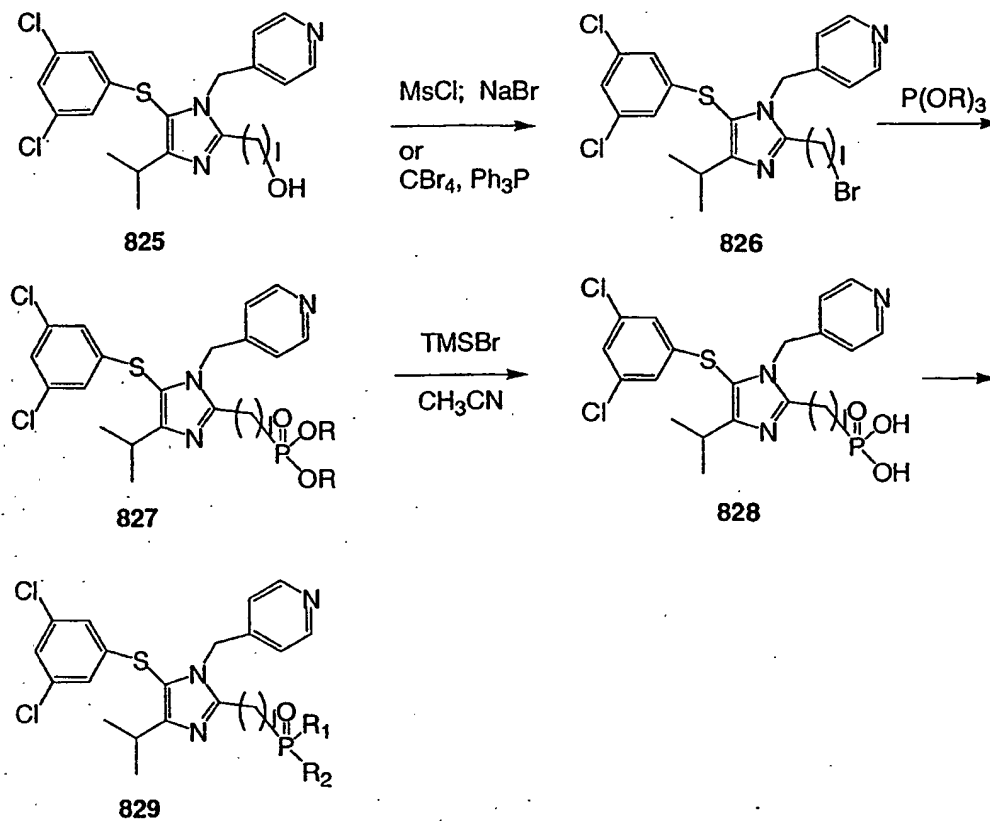
Treatment of alcohol **801** (prepared according to literature) with MsCl and TEA afforded chloride **802** (Scheme 7). Chloride **802** was converted to compound **803** by reacting with **809**, which preparation is detailed in Schemes 3 and 4, in the presence of base. When mesylate **802** was treated with NaCN , imidazole nitrile **804** was provided. Reduction of **804** with DIBAL followed by NaBH_4 yielded imidazole alcohol **806**. Repeating the same procedure several times furnished alcohol **807** with the desired length. Hydrolysis of imidazole nitrile **804** provided acid **805**. Coupling of acid **805** in the presence of conventional reagents afforded the amide **808**. Phosphorus compound **807'** was produced by transforming alcohol **807** to its corresponding mesylate followed by treating with amine **809**.

Scheme 7



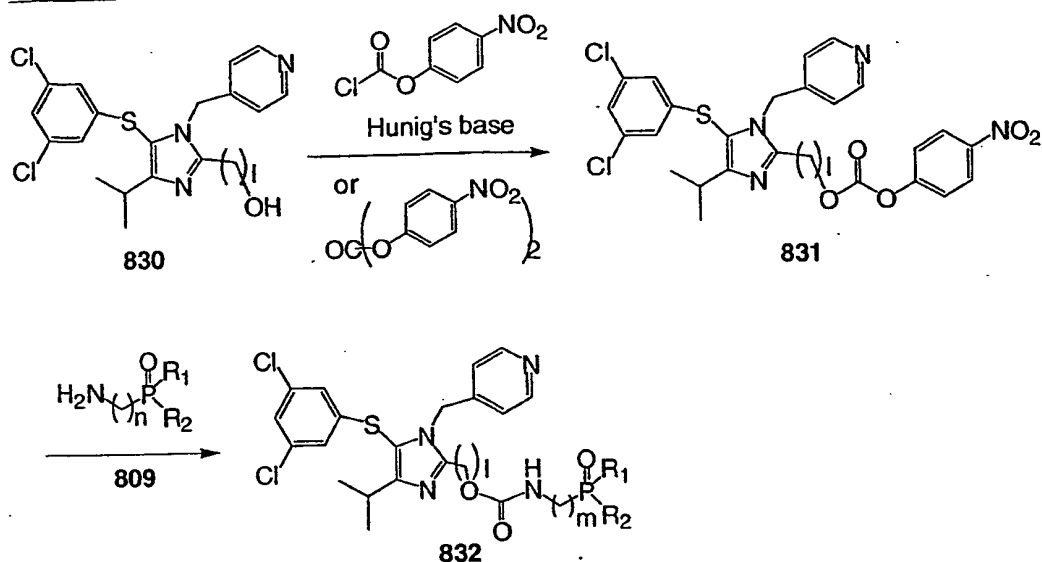
Alcohol 825 was converted to bromide 826 by first transformed to its mesylate and then treated with NaBr, this conversion was also realized by reacting alcohol 825 with Ph_3P and CBr_4 (Scheme 8). Upon treating with $\text{P}(\text{OR})_3$, phosphonate 827 was produced. Esters was then removed to form acid, and following the similar procedure described in Scheme 2 and 3, desired phosphonate, bisphosphoamidate, mono-phosphoamidate, and monophospholactate were produced.

Scheme 8



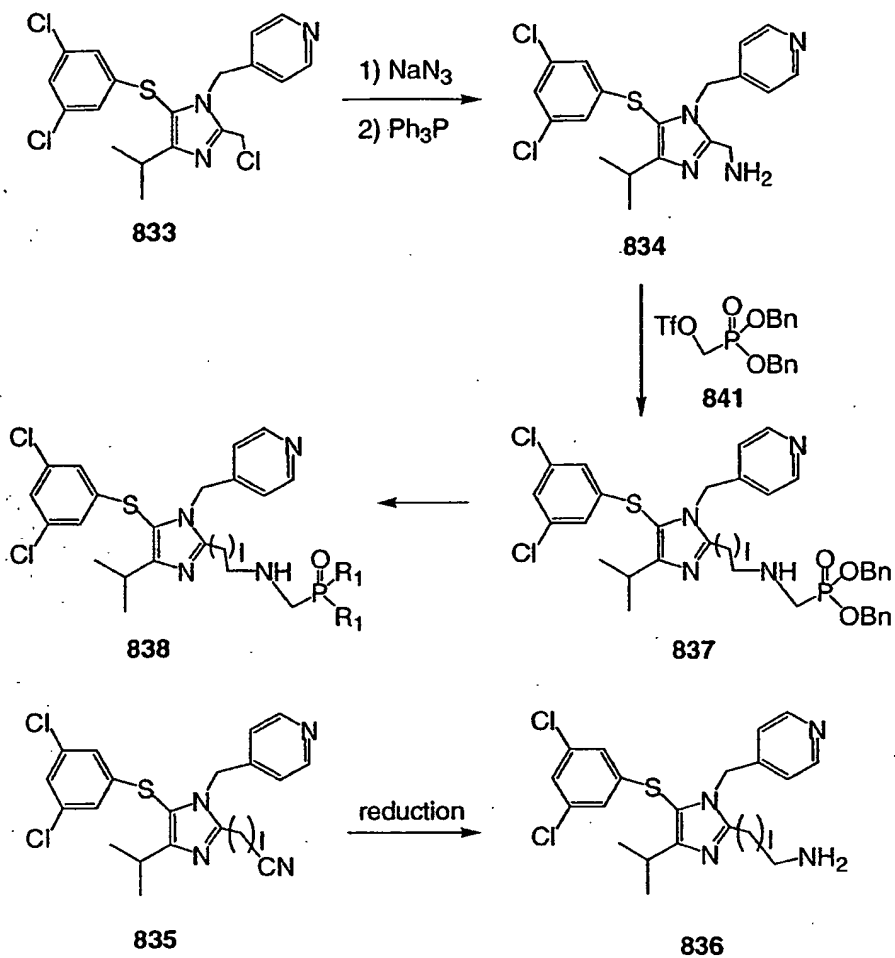
In Scheme 9, alcohol 830 was converted to carbonate 831 by reacting with either p-nitrophenyl chloroformate or p-nitrophenyl carboxy anhydride. Treatment of carbonate 831 with amine 809 in the presence of suitable base afforded desired phosphonate compounds 832.

Scheme 9



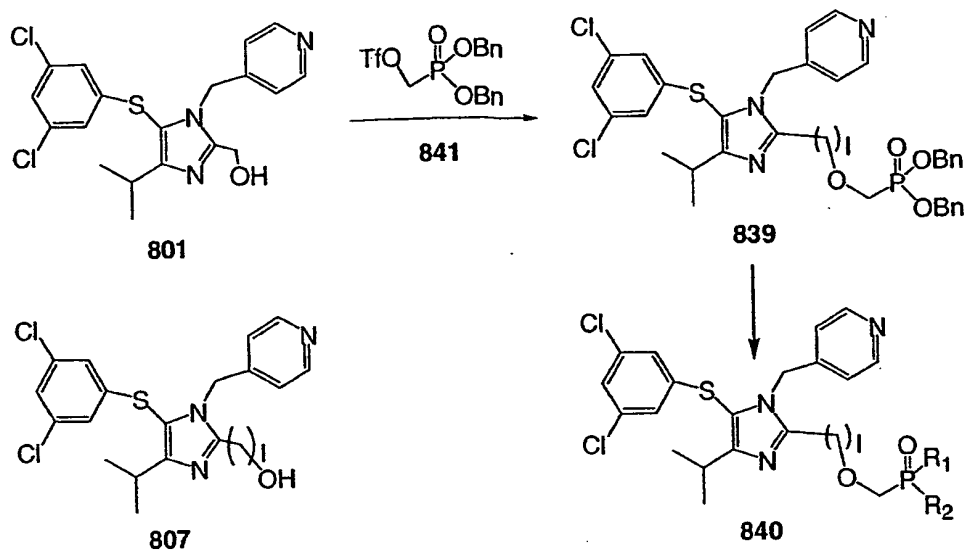
Phosphorus compound 838 was produced according to the procedures described in Scheme 10. Replacement of chloride group in compound 833 with azide followed by reduction with triphenylphosphine provided amine 834. Replacement of chloride group in compound 833 with cyanide, e.g. sodium cyanide, provided amine 835. Reduction of nitrile 835 furnished amine 836. Reaction of amines, e.g. 834 or 836, with triflate 841 in the presence of a base afforded phosphonate 837. Removal of benzyl group of 837 gave its corresponding phosphonic acid, e.g. 838 where $\text{R}_1 = \text{H}$, which was converted to various phosphorus compounds according to the procedure described in the previous Schemes.

Scheme 10



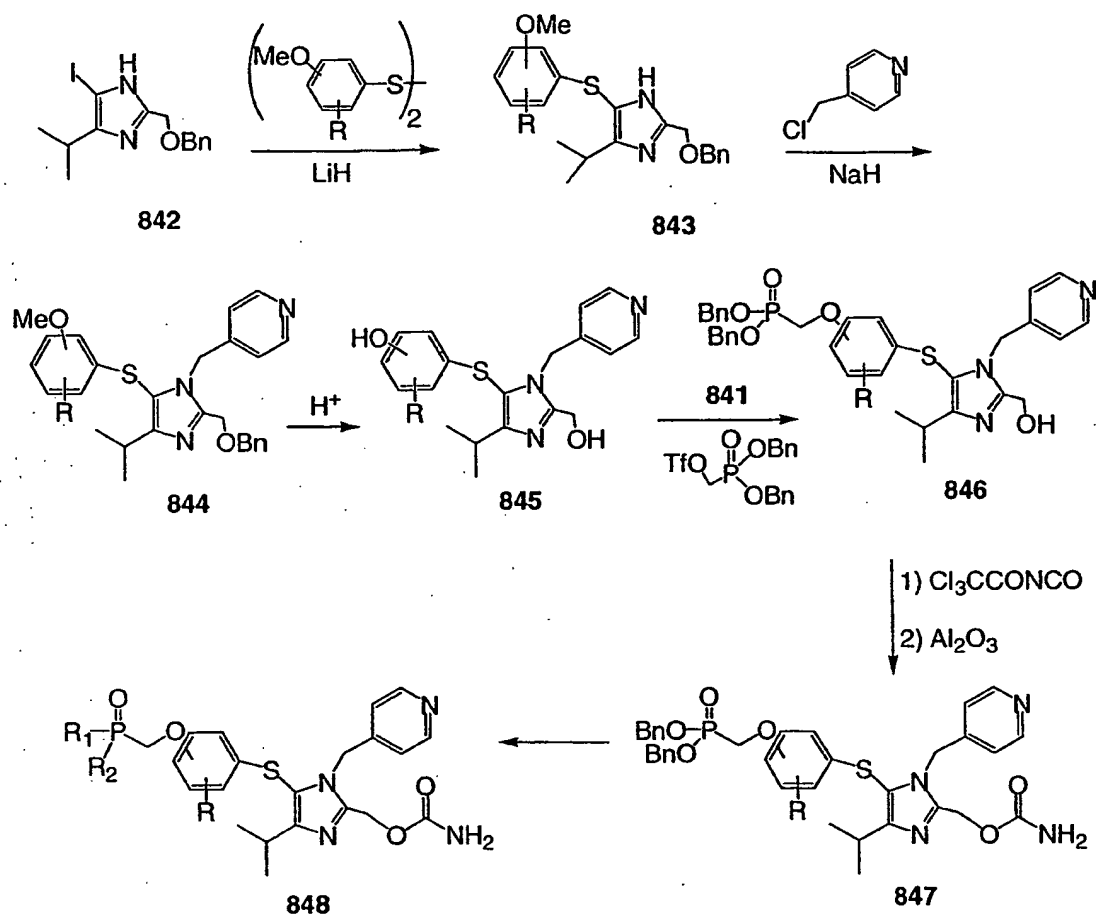
Phosphorus compound 840 was produced in a similar way as described in Scheme 10 except by replacing amines with alcohols 801, or generally, 807 (Scheme 11).

Scheme 11



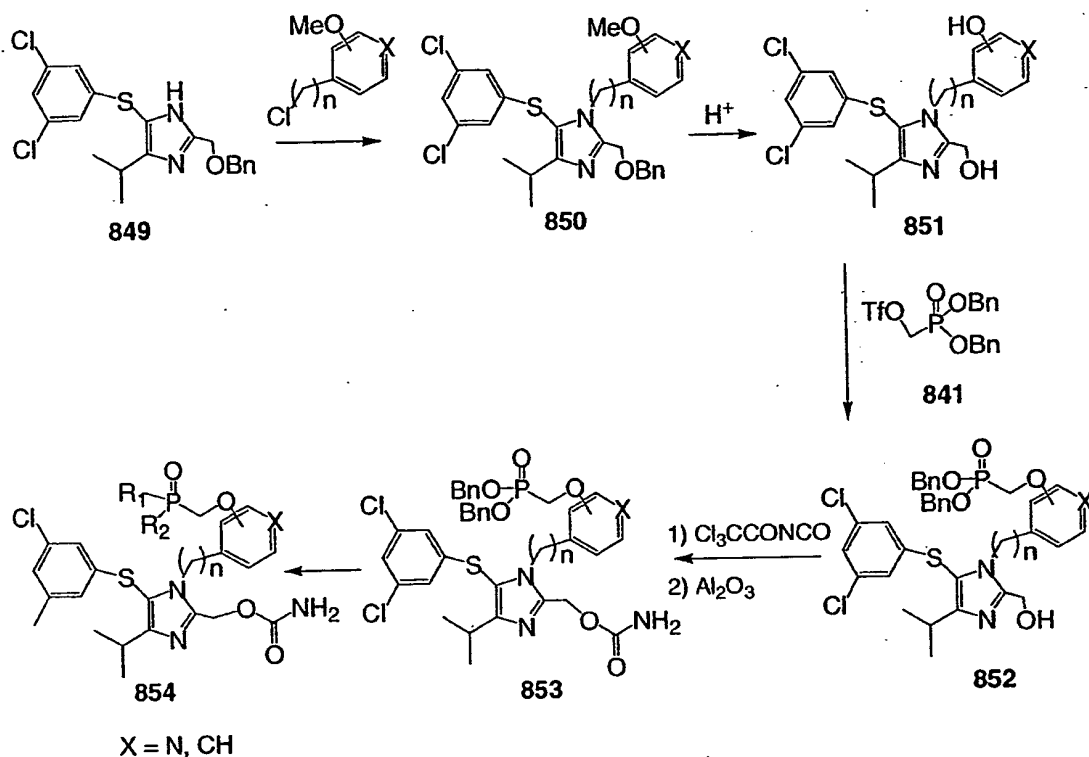
Phosphorus compound **848** was synthesized according to procedures described in Scheme 12. Iodoimidazole **842** was converted to imidazole phenyl thioether **843** by reacting with LiH and substituted phenyl disulfide (Scheme 12). Treatment of imidazole with NaH and 4-picolyl chloride gave imidazole **844**. Benzyl and methyl groups were removed by treating with strong acid to provide alcohol **845**. Conversion of phenol **845** to phosphonate **846** was accomplished by reacting phenol **845** with triflate **841** in the presence of base. Alcohol **846** was reacting with trichloroacetyl isocyanate followed by treatment of alumina afforded carbamate **847**. Phosphonate **847** was transformed to all kinds of phosphorus compound **848** followed the procedure described for **838** in Scheme 10.

Scheme 12



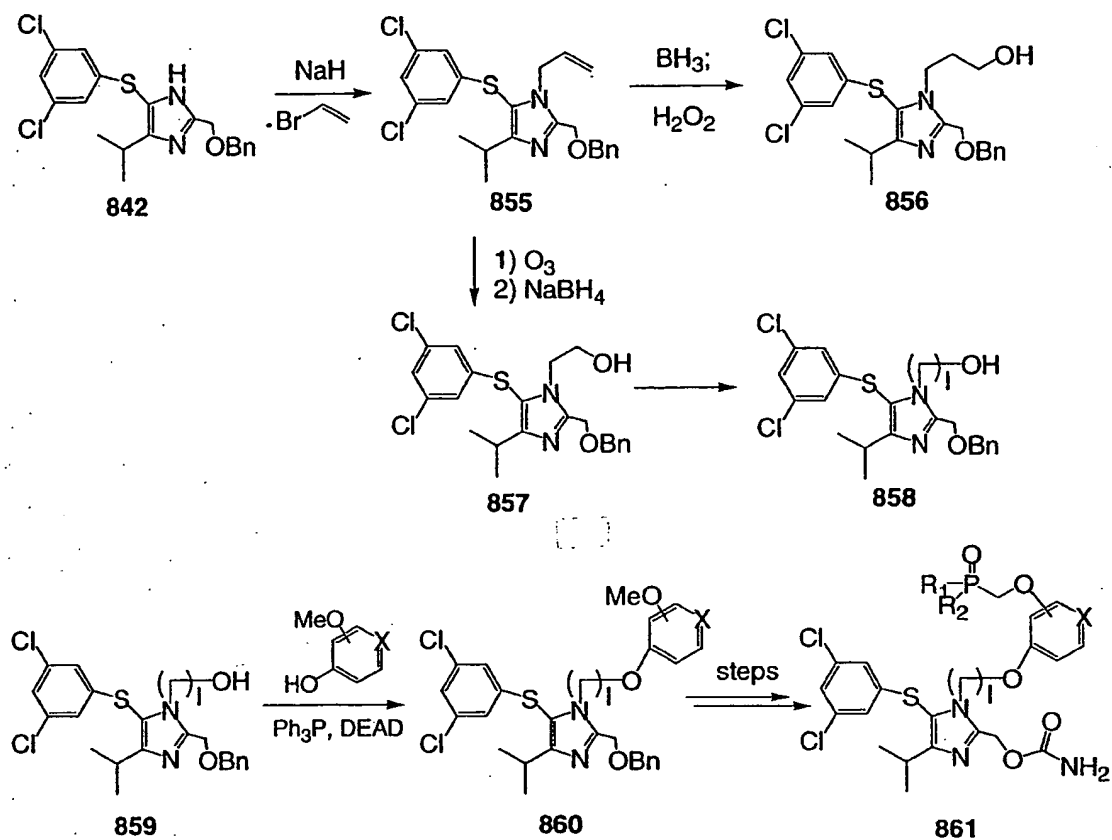
Phosphorus compound 854 was prepared as shown in Scheme 13. Imidazole 849 (prepared according to US Patent Nos. 5910506 and 6057448) was converted to 850 by reacting with chloride in the presence of base. Benzyl and methyl groups were removed by treating ether 850 with strong protonic or Lewis acid to furnish phenol 851. Treatment of phenol 851 with base followed by triflate 841 gave phosphonate 852. Following similar procedures described in Scheme 12 transforming alcohol 846 to phosphorus compound 848, alcohol 852 was converted to phosphorus compound 854.

Scheme 13

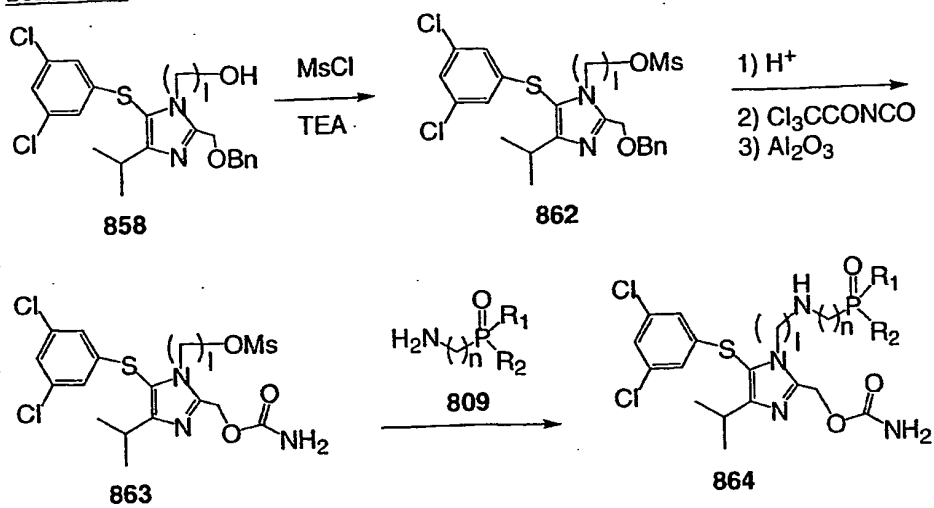


Preparation of phosphorus compound **861** is shown in Scheme 14. Imidazole **855** was synthesized by treating compound **842** with NaH followed by allyl bromide. Hydroboration followed by oxidative work up gave alcohol **856**. Ozonolysis followed by reduction of the resulting aldehyde afforded alcohol **857**. Alcohol **858**, which has variation of length, was obtained by following the same transformation of alcohol **806** to **807** as exhibited in Scheme 7. Mitsunobu reaction of alcohol **859** with substituted phenols gave imidazole **860**. Phenol ether **860** was converted to phosphonate **861** by following same procedure of transforming compound **850** to **854** as described in Scheme 13.

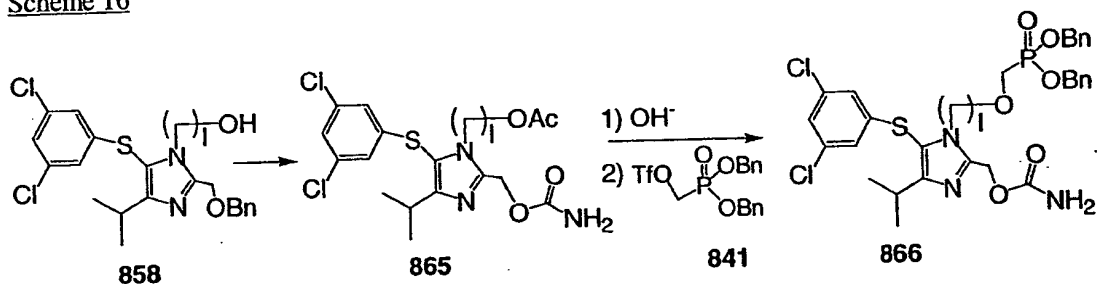
Scheme 14



In Scheme 15, preparation of phosphorus compounds 864 is shown. Alcohol 858 was converted to mesylate 862 by reacting with MsCl . Removal of benzyl group, followed by conversion of the resultant alcohol to the corresponding carbamate (described in previous Schemes) furnished compound 863. Substitution of mesylate with amine 809 generated phosphorus compound 864.

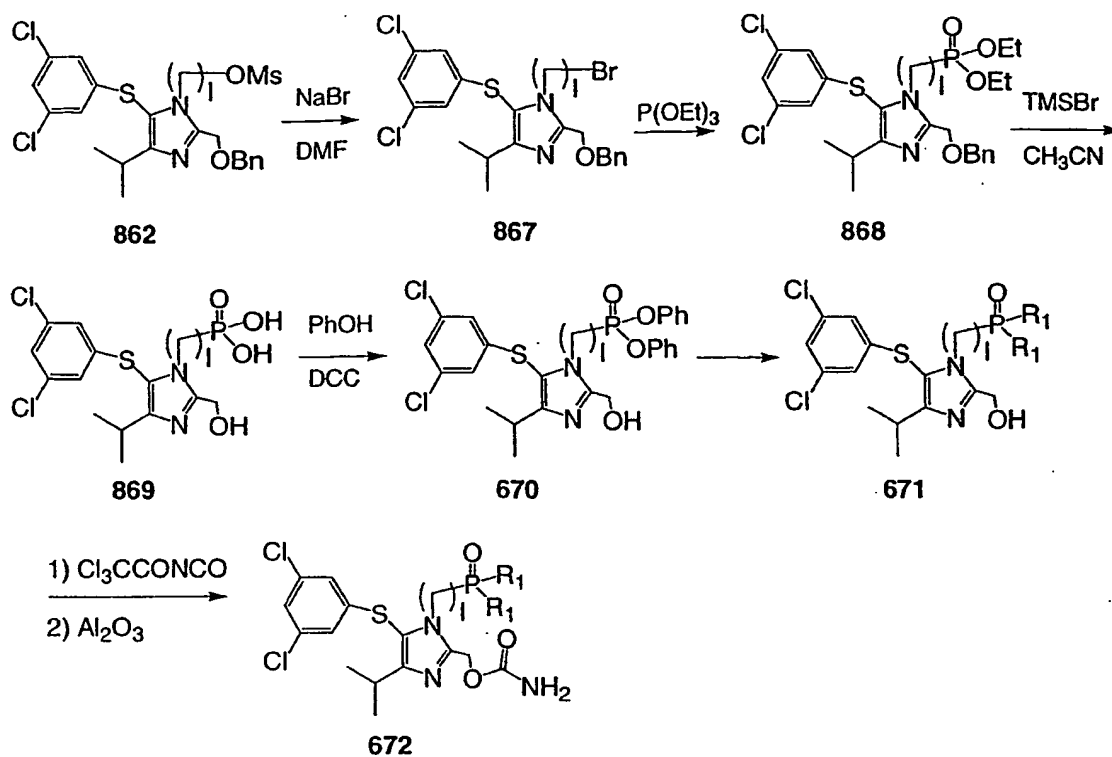
Scheme 15

Synthesis of phosphorus compound 866 is described in Scheme 16. Protection of alcohol 858 to its acetate 865, followed by the conversion of the benzyl, —OBn group to the corresponding carbamate as described for transforming compound 862 to 863 in Scheme 15, gave compound 865. Hydrolysis of acetate, and treatment of the resultant alcohol with triflate 841 in the presence of base afforded phosphonate 866.

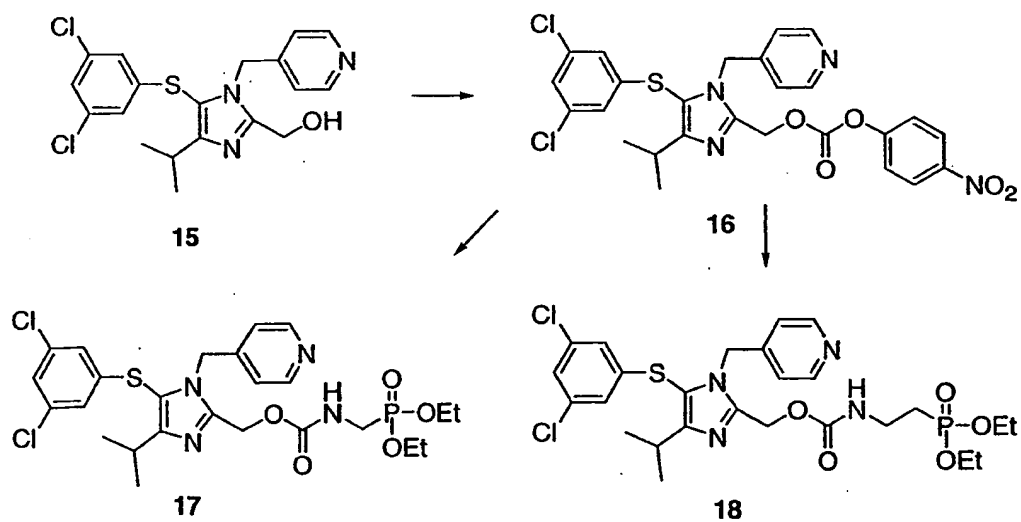
Scheme 16

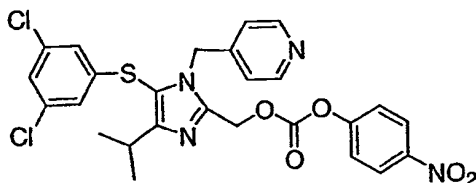
Scheme 17 describes synthesis of phosphorus compound 672. Mesylate 862 was transformed to bromide 867 by reacting with NaBr. Arbusov reaction gave phosphonate 868. Both benzyl and ethyl groups were cleaved when treated with TMSBr to yield compound 869. Coupling of phosphonic acid 869 with PhOH provided bisphenyl phosphonate 670. Compound 670 was converted to various phosphorus compounds 671 according to the procedures described in Schemes 1, 2 and 3. Phosphorus compound 672 was obtained by repeating the procedures shown before.

Scheme 17

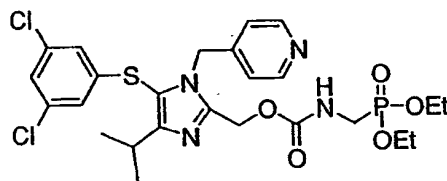


Scheme 18

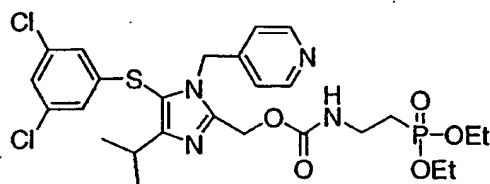


Example 10**16**

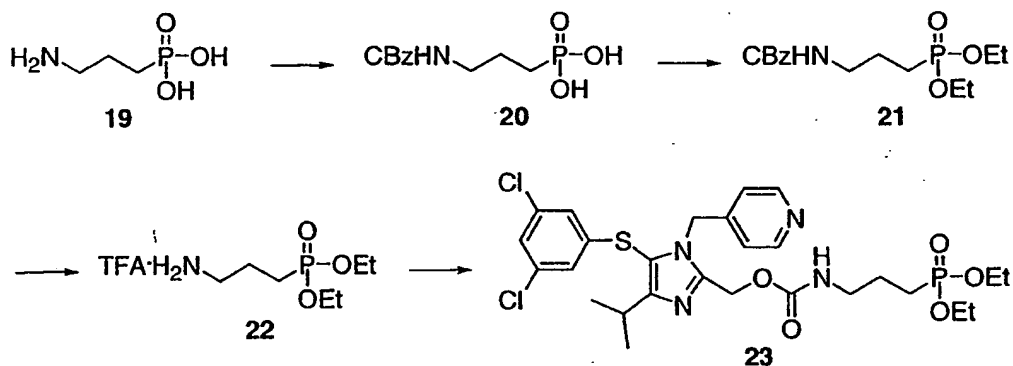
To a solution of alcohol **15** (42 mg, 0.10 mmol) in CH_2Cl_2 (5 mL) was added triethylamine (24 μL , 0.17 mmol) and bis(4-nitrophenyl) carbonate (46 mg, 0.15 mmol). See Scheme 18. After the reaction mixture was stirred for 4 h at room temperature, the mixture was partitioned between CH_2Cl_2 and water. The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 60-70% EtOAc/hexane) to give carbonic acid 5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethyl ester 4-nitro-phenyl ester **16** (47 mg, 82%) as a colorless oil.

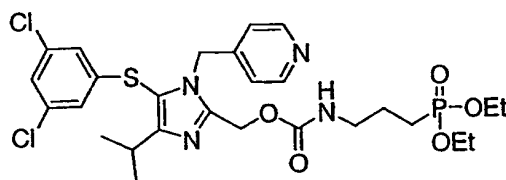
Example 11A**17**

To a solution of carbonate **16** (14 mg, 0.024 mmol) in CH_3CN (2 mL) was added diethyl(aminomethyl)phosphonate (10 mg, 0.037 mmol) and diisopropylethylamine (8 μL , 0.048 mmol). See Scheme 18. After the reaction mixture was stirred for 16 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (eluting 5% MeOH/ CH_2Cl_2) to give ([5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-methyl}-phosphonic acid diethyl ester **17** (13 mg, 90%) as a pale yellow oil. ^1H NMR (300 MHz, CDCl_3) δ 8.44 (d, 2H), 7.04 (t, 1H), 6.78 (d, 2H), 6.68 (d, 2H), 5.25 (s, 2H), 5.19 (s, 2H), 4.98 (bt, 1H), 4.11 (dq, 4H), 3.49 (ABq, 2H), 3.17 (dq, 1H), 1.30 (m, 12H). ^{31}P NMR (300 MHz, CDCl_3) δ 21.9.

Example 11B**18**

To a solution of carbonate **16** (82 mg, 0.143 mmol) in CH_3CN (5 mL) was added diethyl(aminoethyl)phosphonate (58 mg, 0.214 mmol) and diisopropylethylamine (0.05 mL, 0.286 mmol). See Scheme 20. After the reaction mixture was stirred for 16 h at room temperature, the mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (eluting 5-7.5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give 2-[5-(3,5-Dichlorophenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-ethyl}-phosphonic acid diethyl ester **18** (79 mg, 90%) as a pale yellow oil. ^1H NMR (300 MHz, CDCl_3) δ 8.43 (d, 2H), 7.02 (s, 1H), 6.77 (d, 2H), 6.67 (s, 2H), 5.32 (t, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.08 (m, 4H), 3.35 (m, 2H), 3.15 (m, 1H), 1.86 (m, 2H), 1.30 (m, 6H), 1.29 (s, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 31.5.

Scheme 19

Example 11C**23**

To a solution of 3-aminopropylphosphonic acid **19** (500 g, 3.59 mmol) in 2N NaOH (3.6 mL, 7.19 mmol) was added benzyl chloroformate (0.62 mL, 4.31 mmol) according to Scheme 19. After the reaction mixture was stirred for 16 hours at room temperature, the mixture was partitioned between Et₂O and water. The aqueous phase was acidified with 6N HCl until pH = 2. The resulting colorless solid was dissolved in MeOH (75 mL) and treated with Dowex 50WX8-200 (2.5 g). After the mixture was stirred for 30 minutes, it was filtered and evaporated under reduced pressure to give carbamate **20** (880 mg, 90%) as a colorless solid.

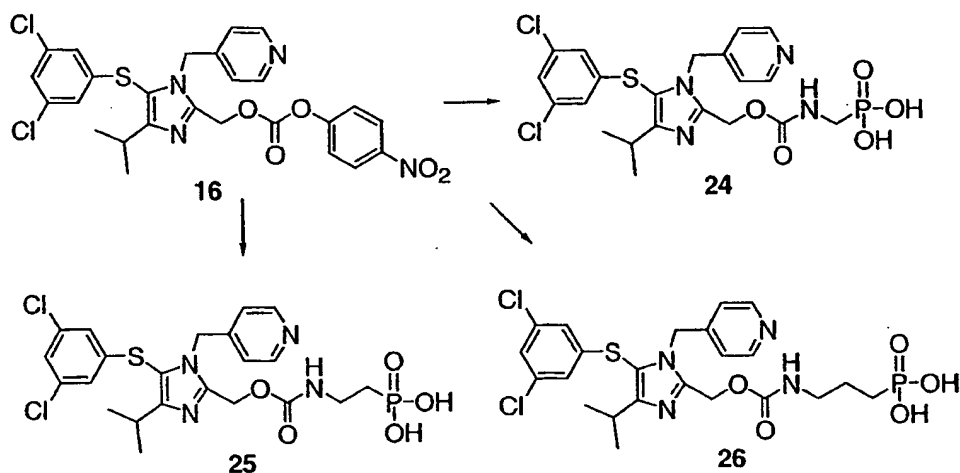
To a solution of carbamate **20** (246 mg, 0.90 mmol) in benzene (5 mL) was added 1,8-diazabicyclo[5.4.0]undec-7-ene phenol (0.27 mL, 1.8 mmol) and iodoethane (0.22 mL, 2.7 mmol). After the reaction mixture was warmed to 60°C and stirred for 16 h, the mixture was concentrated under reduced pressure and partitioned between EtOAc and sat. NH₄Cl. The crude product was chromatographed on silica gel (eluting 3-4% MeOH/CH₂Cl₂) to give phosphonate **21** (56 mg, 19%) as a colorless oil.

To a solution of phosphonate **21** (56 mg, 0.17 mmol) in EtOH (3 mL) was added TFA (13 µL, 0.17 mmol) and 10% Pd/C (11 mg). After the reaction mixture was stirred under H₂ atmosphere (balloon) for 1 h, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give amine **22** (52 mg, 99%) as a colorless oil.

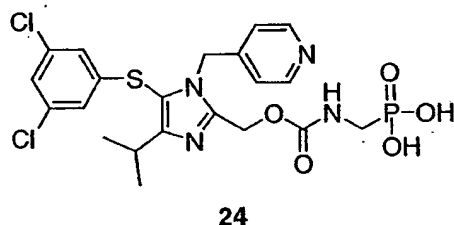
To a solution of carbonate **16** (15 mg, 0.026 mmol) in CH₃CN (2 mL) was added diethyl(aminopropyl)phosphonate (16 mg, 0.052 mmol) and diisopropylethylamine (11 µL, 0.065 mmol). After the reaction mixture was stirred for 16 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by preparative thin layer chromatography (eluting 5% MeOH/CH₂Cl₂) to give {3-[5-(3,5-dichlorophenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-propyl}-phosphonic acid diethyl ester **23** (13 mg, 79%) as a pale yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 8.44 (d, 2H), 7.04 (t, 1H), 6.80 (d, 2H), 6.68 (d, 2H), 5.26 (s, 2H), 5.18 (s,

2H), 5.08 (bt, 1H), 4.08 (m, 4H), 3.15 (m, 3H), 1.72 (m, 4H), 1.31 (m, 12H). ^{31}P NMR (300 MHz, CDCl_3) δ 31.5.

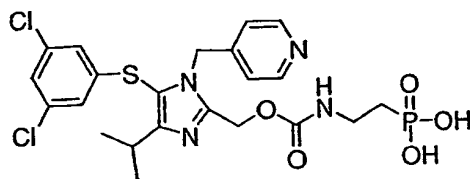
Scheme 20



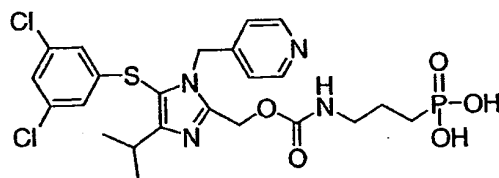
Example 12A



To a solution of aminomethylphosphonic acid (8 mg, 0.073 mmol) in water (1 mL) was added 1N NaOH (0.15 mL, 0.15 mmol) and carbonate 16 (21 mg, 0.037 mmol) in dioxane (1 mL). See Scheme 20. After the reaction mixture was stirred for 6 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by HPLC on C18 reverse phase chromatography (eluting 30% CH_3CN /water) to give a mixture of phosphonic acid 24 and alcohol 15. The mixture was further purified by preparative thin layer chromatography (eluting 7.5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give {[5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonyl amino]-methyl}-phosphonic acid 24 (8 mg, 40%) as a colorless solid. ^1H NMR (300 MHz, CD_3OD) δ 8.33 (bs, 2H), 7.10 (t, 1H), 7.04 (bs, (2H), 6.72 (d, 2H), 5.44 (s, 2H), 5.25 (s, 2H), 3.24 (m, 2H), 3.17 (m, 1H), 1.28 (d, 6H).

Example 12B**25**

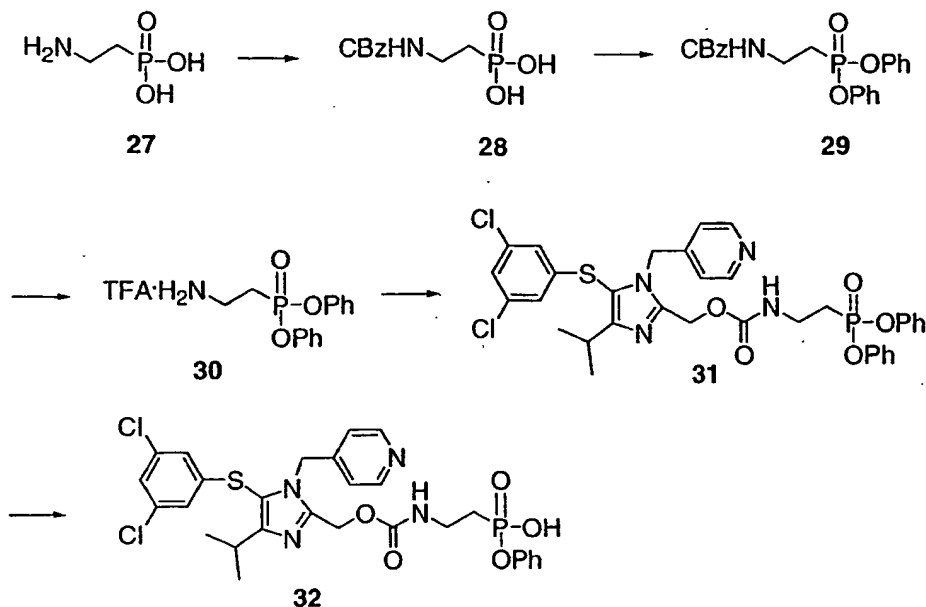
To a solution of 2-aminoethylphosphonic acid (12 mg, 0.098 mmol) in water (1 mL) was added 1N NaOH (0.2 mL, 0.20 mmol) and carbonate **16** (28 mg, 0.049 mmol) in dioxane (1 mL). See Scheme 20. After the reaction mixture was stirred for 6 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by HPLC on C18 reverse phase chromatography (eluting 30% CH₃CN/water) to give a mixture of phosphonic acid **25** and alcohol **15**. The mixture was further purified by preparative thin layer chromatography (eluting 7.5% MeOH/CH₂Cl₂) to give {2-[5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-ethyl}-phosphonic acid **25** (13 mg, 47%) as a colorless solid. ¹H NMR (300 MHz, CD₃OD) δ 8.32 (d, 2H), 7.11 (s, 1H), 7.02 (d, 2H), 6.72 (s, 2H), 5.42 (s, 2H), 5.23 (s, 2H), 3.30 (m, 2H), 3.17 (m, 1H), 1.71 (m, 2H), 1.28 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 20.1.

Example 12C**26**

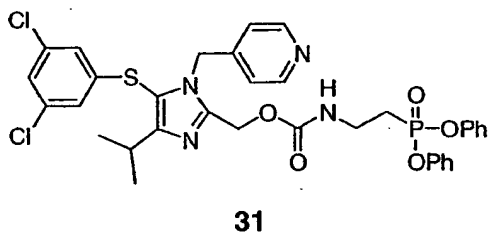
To a solution of 3-aminopropylphosphonic acid (12 mg, 0.084 mmol) in water (1 mL) was added 1N NaOH (0.17 mL, 0.17 mmol) and carbonate **16** (24 mg, 0.042 mmol) in dioxane (1 mL). See Scheme 20. After the reaction mixture was stirred for 6 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified by HPLC on C18 reverse phase chromatography (eluting 30% CH₃CN/water) to give a mixture of phosphonic acid **26** and alcohol **15**. The mixture was further purified by preparative thin layer chromatography (eluting 7.5% MeOH/CH₂Cl₂) to give {3-[5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-propyl}-phosphonic acid **26** (11 mg, 46%) as a colorless solid. ¹H

NMR (300 MHz, CD₃OD) δ 8.34 (bs, 2H), 7.11 (s, 1H), 7.02 (bs, 2H), 6.73 (d, 2H), 5.43 (s, 2H), 5.23 (s, 2H), 3.32 (m, 1H), 3.06 (bs, 2H), 1.69 (bs, 2H), 1.50 (bs, 2H), 1.28 (d, 6H).

Scheme 21



Example 13



To a solution of 2-aminoethylphosphonic acid (1.26 g, 10.1 mmol) in 2N NaOH (10.1 mL, 20.2 mmol) was added benzyl chloroformate (1.7 mL, 12.1 mmol). See Scheme 21. After the reaction mixture was stirred for 2 d at room temperature, the mixture was partitioned between Et₂O and water. The aqueous phase was acidified with 6N HCl until pH = 2. The resulting colorless solid was dissolved in MeOH (75 mL) and treated with Dowex 50WX8-200 (7 g). After the mixture was stirred for 30 minutes, it was filtered and evaporated under reduced pressure to give carbamate **28** (2.37 g, 91%) as a colorless solid.

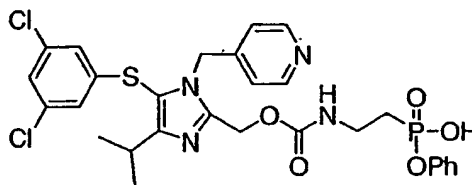
To a solution of carbamate **28** (2.35 g, 9.1 mmol) in pyridine (40 mL) was added phenol (8.53 g, 90.6 mmol) and 1,3-dicyclohexylcarbodiimide (7.47 g, 36.2 mmol). After the reaction mixture was warmed to 70°C and stirred for 5 h, the mixture was diluted with

CH₃CN and filtered. The filtrate was concentrated under reduced pressure and diluted with EtOAc. The organic phase was washed with sat. NH₄Cl, sat. NaHCO₃, and brine, then dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel twice (eluting 40-60% EtOAc/hexane) to give phosphonate **29** (2.13 g, 57%) as a colorless solid.

To a solution of phosphonate **29** (262 mg, 0.637 mmol) in isopropanol (iPrOH) (5 mL) was added TFA (0.05 mL, 0.637 mmol) and 10% Pd/C (26 mg). After the reaction mixture was stirred under H₂ atmosphere (balloon) for 1 h, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give amine **30** (249 mg, 100%) as a colorless oil.

To a solution of carbonate **16** (40 mg, 0.070 mmol) and amine **30** (82 mg, 0.21 mmol) in CH₃CN (5 mL) was added diisopropylethylamine (0.05 mL, 0.28 mmol). After the reaction mixture was stirred for 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (eluting 3-4% MeOH/CH₂Cl₂) to give {2-[5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-ethyl}-phosphonic acid diphenyl ester **31** (36 mg, 72%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 8.37 (d, 2H), 7.22 (m, 4H), 7.14 (m, 2H), 7.10 (m, 2H), 6.99 (t, 1H), 6.72 (d, 2H), 6.62 (d, 2H), 5.30 (bt, 1H), 5.18 (s, 2H), 5.13 (s, 2H), 3.50 (m, 2H), 3.12 (m, 1H), 2.21 (m, 2H), 1.26 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 22.4.

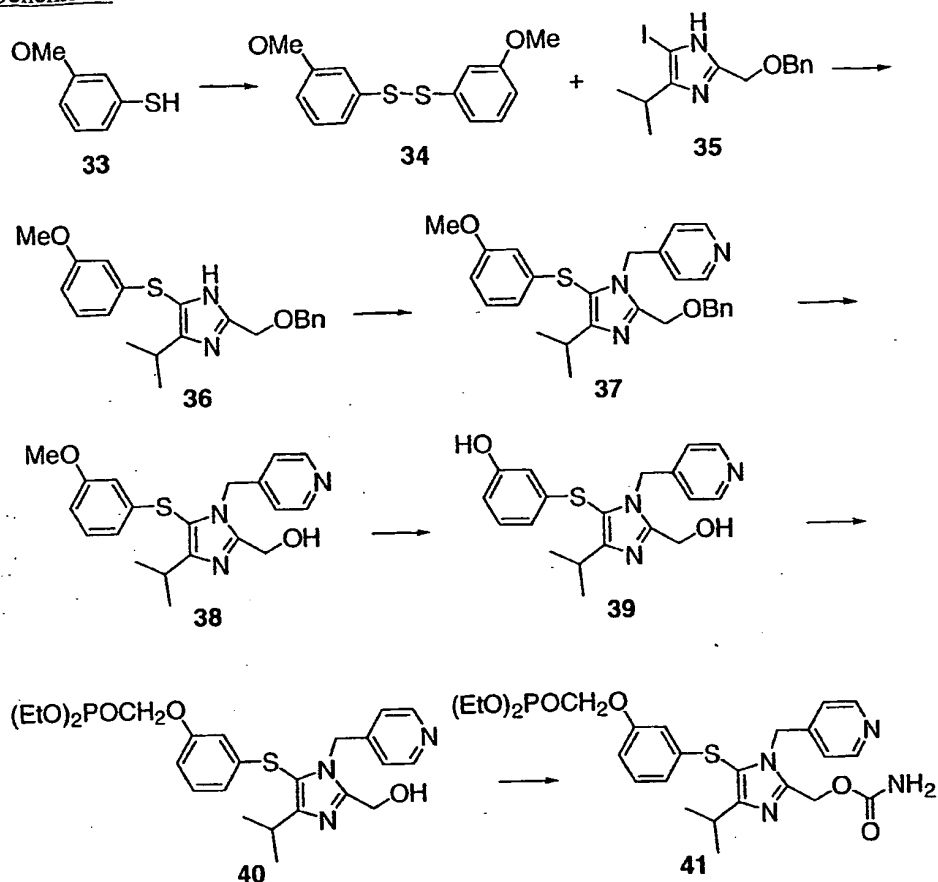
Example 14



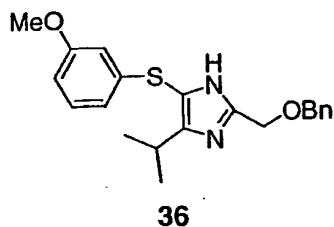
32

To a solution of phosphonate **31** (11 mg, 0.015 mmol) in CH₃CN (0.5 mL) was added 1N LiOH (46 μL, 0.046 mmol) at 0°C. See Scheme 21. After the reaction mixture was stirred for 2 h at 0°C, Dowex 50WX8-200 (26 mg) was added and stirring was continued for an additional 30 min. The reaction mixture was filtered, rinsed with CH₃CN, and concentrated under reduced pressure to give {2-[5-(3,5-dichloro-phenylsulfanyl)-4-isopropyl-1-pyridin-4-ylmethyl-1H-imidazol-2-ylmethoxycarbonylamino]-ethyl}-phosphonic acid monophenyl ester **32** (10 mg, 100%) as a colorless oil. ¹H NMR (300 MHz, CD₃OD) δ 8.52 (d, 2H), 7.28 (m, 6H), 6.79 (m, 4H), 5.60 (s, 2H), 5.29 (s, 2H), 3.29 (m, 3H), 1.83 (m, 2H), 1.31 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 20.2.

Scheme 22



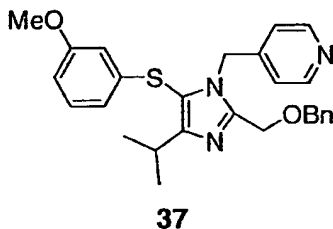
Example 15



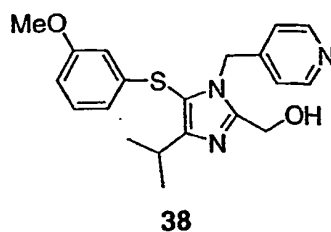
To a solution of 3-methoxybenzenethiol (0.88 mL, 7.13 mmol) in CH₃CN (15 mL) was added sodium iodide (214 mg, 1.43 mmol) and ferric chloride (232 mg, 1.43 mmol). See Scheme 22. After the reaction mixture was warmed to 60°C and stirred for 3 d, the mixture was concentrated under reduced pressure and partitioned between CH₂Cl₂ and water. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The

crude product was chromatographed on silica gel (eluting 5-6% EtOAc/hexane) to give disulfide **34** (851 mg, 86%) as a yellow oil. To a solution of disulfide **34** (850 mg, 3.05 mmol) in DMSO (10 mL) was added iodide **35**, also denoted previously as compound **842**, (1.21 g, 3.39 mmol) and lithium hydride (32 mg, 4.07 mmol). After the reaction mixture was warmed to 60°C and stirred for 16 h, the mixture was partitioned between EtOAc and water. The organic phase was washed with brine, dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 30-50% EtOAc/hexane) to give 2-benzyloxymethyl-4-isopropyl-5-(3-methoxy-phenylsulfanyl)-1H-imidazole **36** (247 mg, 22%) as a yellow oil.

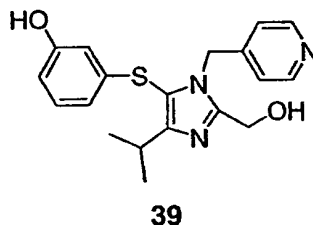
Example 16



To a solution of sulfide **36** (247 mg, 0.67 mmol) in THF (10 mL) was added 4-picolylchloride (220 mg, 1.34 mmol), powder NaOH (59 mg, 1.47 mmol), lithium iodide (44 mg, 0.33 mmol), and tetrabutylammonium bromide (22 mg, 0.067 mmol). See Scheme 22. After the reaction mixture was stirred for 2 d at room temperature, the mixture was partitioned between EtOAc and sat. NH₄Cl. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 60-100% EtOAc/hexane) to give 4-[2-benzyloxymethyl-4-isopropyl-5-(3-methoxy-phenylsulfanyl)-imidazol-1-ylmethyl]-pyridine **37** (201 mg, 65%) as a yellow oil.

Example 17

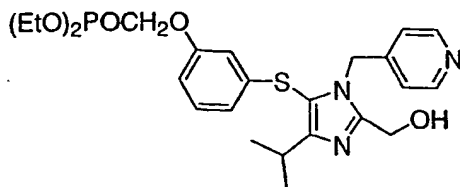
5 To a solution of amine 37 (101 mg, 0.220 mmol) in EtOH (5 mL) was added conc. HCl (5 mL). See Scheme 22. After the reaction mixture was warmed to 80°C and stirred for 16 h, the mixture was concentrated under reduced pressure and partitioned between EtOAc and sat. NaHCO₃. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 5-7% MeOH/CH₂Cl₂) to give [4-isopropyl-5-(3-methoxy-phenylsulfanyl)-1-pyridin-4-ylmethyl-1H-imidazol-2-yl]-methanol **38** (71 mg, 87%) as a pale yellow oil.

Example 18

15

To a solution of alcohol **38** (56 mg, 0.15 mmol) in CH₂Cl₂ (2 mL) was added 1M BBr₃ in CH₂Cl₂ at 0°C. See Scheme 22. After the reaction mixture was stirred for 1 h at 0°C, the mixture was partitioned between CH₂Cl₂ and sat. NaHCO₃. The aqueous phase was neutralized with solid NaHCO₃ and extracted with CH₂Cl₂ and EtOAc. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 5-10% MeOH/CH₂Cl₂) to give 3-(2-hydroxymethyl-5-isopropyl-3-pyridin-4-ylmethyl-3H-imidazol-4-ylsulfanyl)-phenol **39** (43 mg, 81%) as a colorless solid.

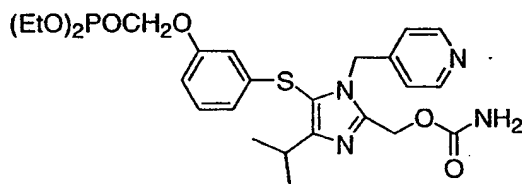
25 Example 19



40

To a solution of phenol **39** (25 mg, 0.070 mmol) and triflate (33 mg, 0.11 mmol) in THF (2 mL) and CH₃CN (2 mL) was added Cs₂CO₃ (46 mg, 0.14 mmol). See Scheme 22. After the reaction mixture was stirred for 1 h at room temperature, the mixture was partitioned between EtOAc and water. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 10% MeOH/CH₂Cl₂) to give [3-(2-Hydroxymethyl-5-isopropyl-3-pyridin-4-ylmethyl-3H-imidazol-4-ylsulfanyl)-phenoxy-methyl]-phosphonic acid diethyl ester **40** (10 mg, 28%) as a colorless oil.

Example 20

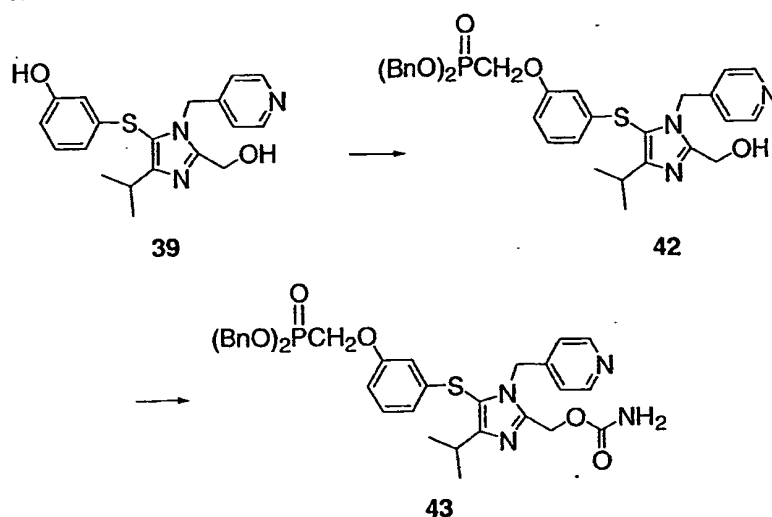


41

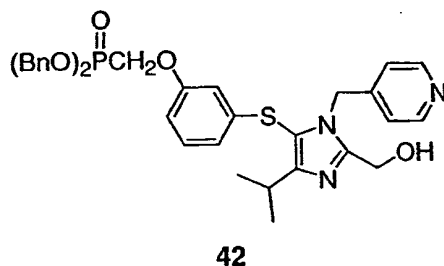
To a solution of diethylphosphonate **40** (10 mg, 0.020 mmol) in THF (2 mL) was added trichloroacetyl isocyanate (7 μ L, 0.059 mmol). See Scheme 22. After the reaction mixture was stirred for 30 min at room temperature, the mixture was evaporated under reduced pressure. To a solution of the concentrated residue in MeOH (2 mL) was added 1M K₂CO₃ (0.2 mL, 0.20 mmol) at 0°C. After the reaction mixture was warmed to room temperature and stirred for 3 h, the mixture was partitioned between EtOAc and sat. NH₄Cl. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 10% MeOH/CH₂Cl₂) to give [3-(2-hydroxymethyl-5-isopropyl-3-pyridin-4-ylmethyl-3H-imidazol-4-ylsulfanyl)-phenoxy-methyl]-phosphonic acid diethyl ester **41** (10 mg, 91%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 8.50 (d, 2H), 7.16 (m, 1H), 6.85 (m, 1H), 6.75 (m, 1H), 6.73

(m, 1H), 6.17 (s, 1H), 5.31 (s, 2H), 5.02 (s, 2H), 4.23 (m, 4H), 4.16 (d, 2H), 3.23 (m, 1H), 1.37 (t, 6H), 1.29 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 19.6.

Scheme 23

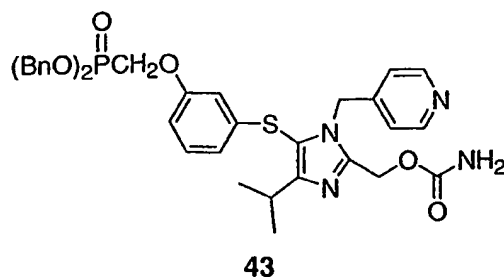


5 Example 21



To a solution of phenol 39 (20 mg, 0.056 mmol) in THF (1 mL) and CH_3CN (1 mL) was added sodium hydride (60%, 5 mg, 0.112 mmol) at 0°C . See Scheme 23. After the reaction mixture was stirred for 30 min at 0°C , dibenzylphosphonyl methyltriflate (21 mg, 0.050 mmol) in THF (1 mL) was added. After the reaction mixture was stirred for 1 h at 0°C , the mixture was evaporated under reduced pressure and partitioned between EtOAc and sat. NH_4Cl . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give dibenzylphosphonate 42 (5 mg, 16%) as a pale yellow oil.

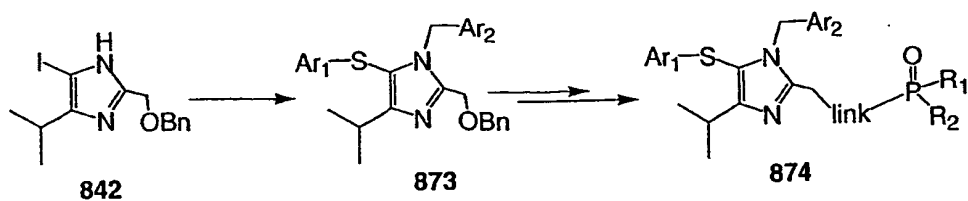
Example 22



To a solution of dibenzylphosphonate **42** (5 mg, 0.0079 mmol) in CH₂Cl₂ (1 mL) was
5 added trichloroacetyl isocyanate (5 μL, 0.049 mmol). See Scheme 23. After the reaction
mixture was stirred for 15 min at room temperature, the mixture was transferred on to a 2-
inch column of neutral Al₂O₃. After the reaction mixture was soaked for 30 min, the mixture
was rinsed off the column with 10% MeOH/CH₂Cl₂ and evaporated under reduced pressure.
The crude product was purified by preparative thin layer chromatography (eluting 10%
10 MeOH/CH₂Cl₂) to give carbamate **43** (3 mg, 56%) as a pale yellow oil. ¹H NMR (300 MHz,
CDCl₃) δ 8.48 (d, 2H), 7.35 (m, 10H), 7.12 (t, 1H), 6.88 (m, 2H), 6.70 (d, 1H), 6.66 (dd,
1H), 6.10 (t, 1H), 5.29 (s, 2H), 5.13 (dd, 6H), 5.05 (s, 2H), 4.14 (d, 2H), 3.24 (m, 1H), 1.30
(d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.3.

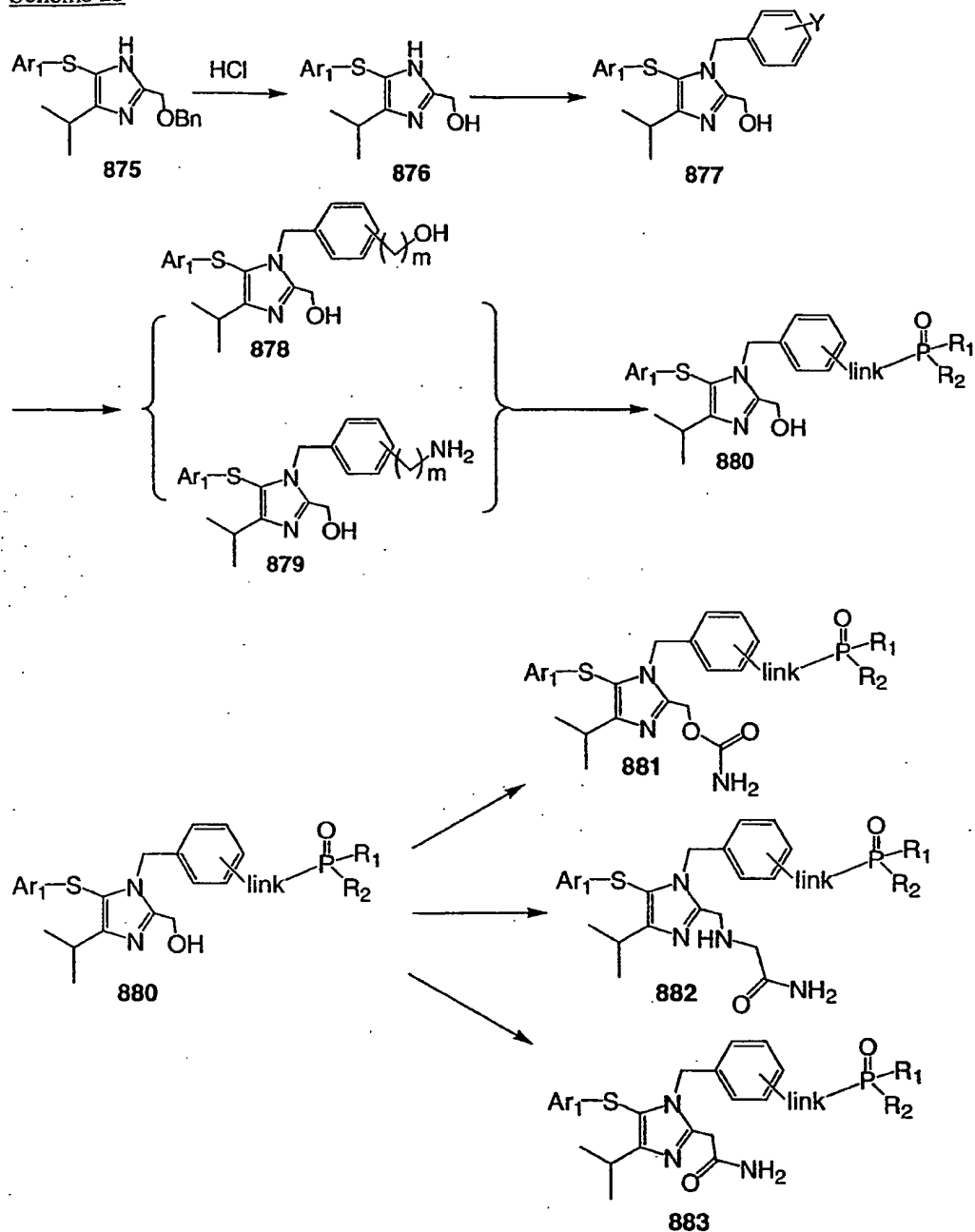
Preparation of phosphorus compound **874** was displayed in Scheme 24. Starting with
15 imidazole **842**, Ar1 and Ar2 were introduced following the procedure described in US Patent
No. 5326780. Benzyl group was then removed and converted to phosphorus analog **874**
using the procedure described previously.

Scheme 24



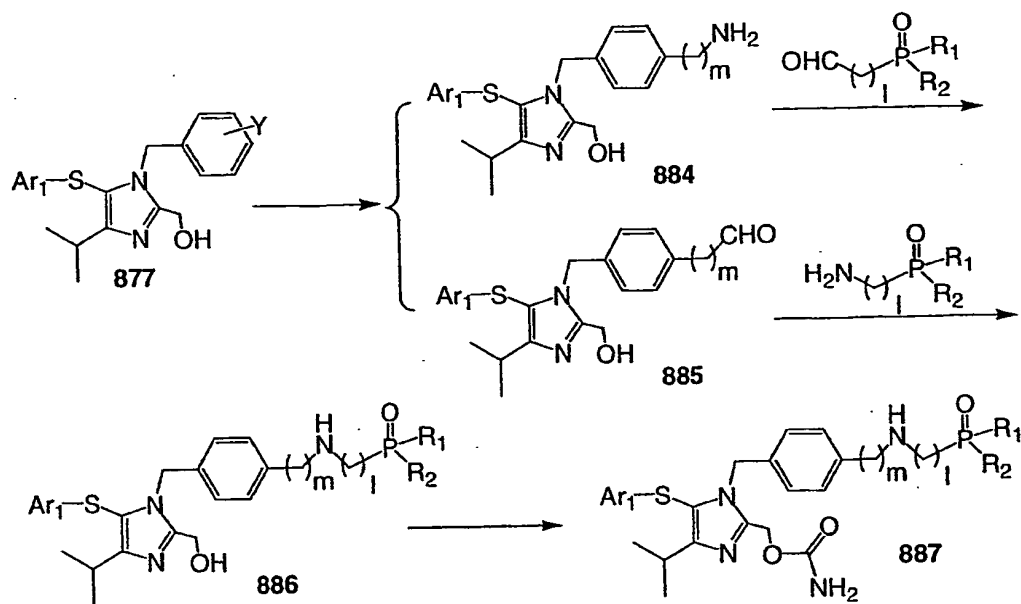
Scheme 25 describes preparation of compound 880. Compound 875 was synthesized from compound 842 using the procedures described in US Patent No. 5326780. Treatment of 875 with HCl removed the benzyl group to give alcohol 876, which was then introduced phenyl group with substitution of Y. Y is a function which can be converted to alcohol, aldehyde or amine, for example -NO₂, -COOMe, N₃, and etc. Conversion of Y to the amine or alcohol gave compound 878 and/or 879, which were then used as attachment site of phosphorus to afford phosphorus compound 880. Hydroxyl group in compound 880 was then converted to the desired side chain including but not limit to carbamate 881, urea 882, substituted amine 883.

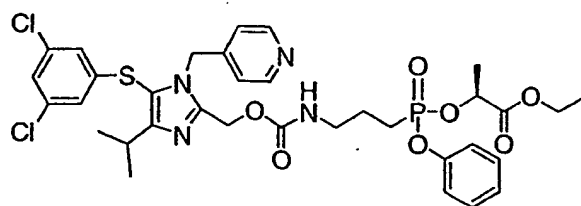
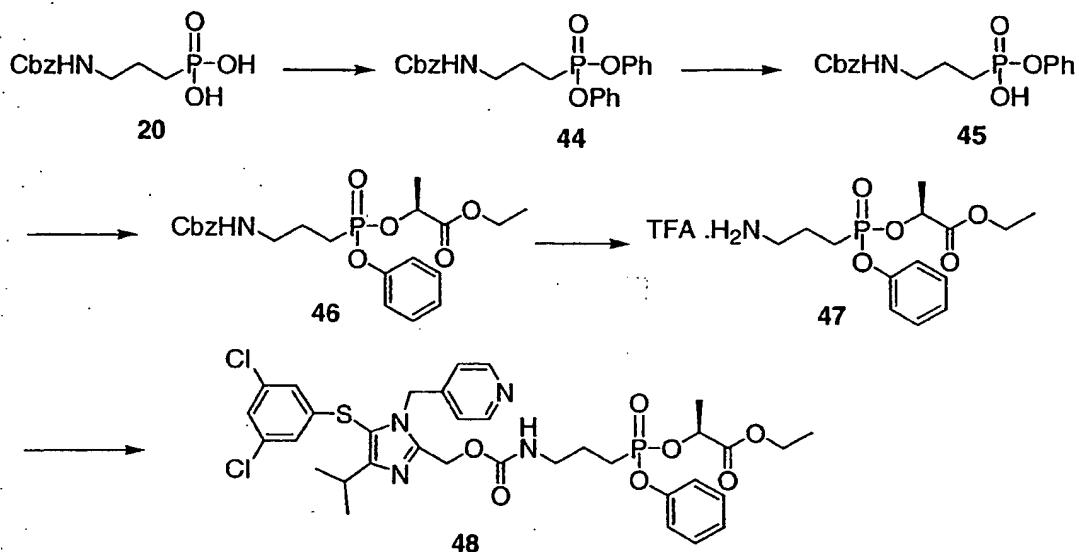
Scheme 25



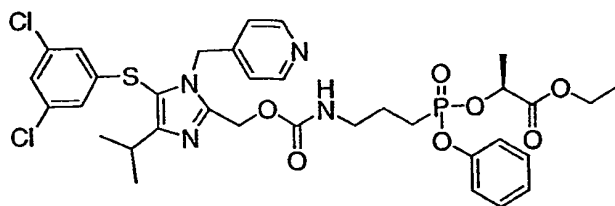
Preparation of phosphorus compound 887 is shown in Scheme 26. Compound 877 was converted to amine 884 and/or aldehyde 885, which then reacted with aldehyde and/or amine respectively to provide phosphorus compound 886. Treatment of compound 886 with Cl_3CCONCO provide the carbamate 887.

Scheme 26

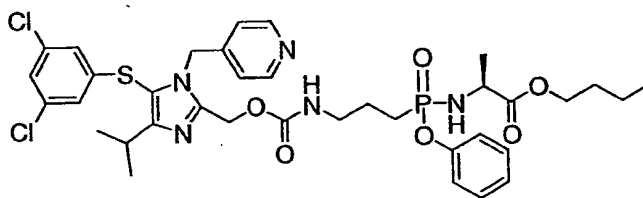
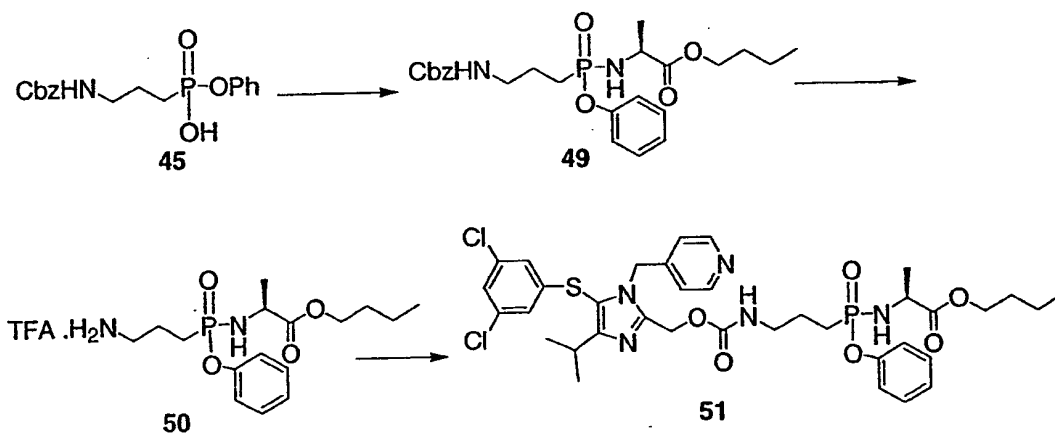


Example 22**48**

Compound 44 was prepared following the sequence of steps described in Example 13, by substituting compound 20 for compound 28. Purification of the crude product on silica gel eluted with 3-4% MeOH/CH₂Cl₂ provided 37 mg of **48**, the title compound. ¹H NMR (500 MHz, CDCl₃) (1.3:1 diastereomeric ratio) δ 8.50 (bs, 2H), 7.35 (t, 2H), 7.20 (m, 3H), 7.06 (s, 1H), 6.90 (bs, 2H), 6.70 (s, 2H), 5.26 (bs, 2H), 5.21 (s, 2H), 4.97 (m, 1H), 4.22 (q, 2H), 3.24 (m, 2H), 3.19 (m, 1H), 2.05 (m, 2H), 1.92 (m, 2H), 1.37 (d, 3H), 1.33 (d, 6H), 1.28 (t, 3H). ³¹P NMR (300 MHz, CDCl₃) δ 30.0.

Example 23**49**

The title compound **49** was prepared following the sequence of steps described in Example 22, except for using scalaric mixture **46** (around 13:1 ratio). Purification of the crude final product on silica gel eluted with 3-4% MeOH/CH₂Cl₂ provided 40 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 8.44 (bd, 2H), 7.32 (m, 2H), 7.19 (m, 3H), 7.04 (d, 1H), 6.80 (bs, 2H), 6.68 (m, 2H), 5.27 (d, 2H), 5.19 (d, 2H), 4.96 (m, 1H), 4.15 (m, 2H), 3.18 (m, 3H), 1.93 (m, 4H), 1.55 (d, 1.5H), 1.34 (d, 1.5H), 1.31 (d, 6H), 1.21 (m, 3H). ³¹P NMR (300 MHz, CDCl₃) δ 30.0, 28.3.

Example 24**51**

Amidate 49: A solution of phosphonic acid **45** (66 mg, 0.19 mmol) in CH₃CN (5 mL) was treated with thionyl chloride (42 μL, 0.57 mmol). After the reaction mixture was warmed

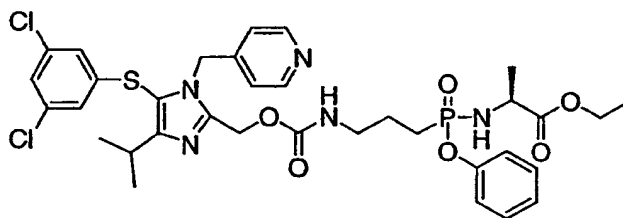
to 70°C and stirred for 2 h, the mixture was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (5 mL) and cooled to 0°C. Triethylamine (0.11 mL, 0.76 mmol) and L-alanine *n*-butyl ester (104 mg, 0.57 mmol) were added. After stirring for 1 h at 0°C and 1 h at room temperature, the reaction mixture was neutralized with sat. NH₄Cl and extracted with CH₂Cl₂ and EtOAc. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 60-80% EtOAc/hexane) to give amidate **49** (35 mg, 39%) as a colorless oil.

Amine 50: A mixture of benzyl carbamate **49** (35 mg, 0.073 mmol), trifluoroacetic acid (8 µL, 0.11 mmol) and 10% Pd/C (7 mg) in isopropyl alcohol (2 mL) was stirred under H₂ atmosphere (balloon) for 1 h. The mixture was then filtered through Celite. The filtrate was evaporated under reduced pressure to give amine **50** (33 mg, 99%) as a colorless oil.

Title compound 51: A solution of 4-nitrophenylcarbonate **16** (35 mg, 0.061 mmol) in CH₃CN (2 mL) was treated with amine **50** (33 mg, 0.072 mmol) and iPr₂NEt (21 µL, 0.122 mmol).

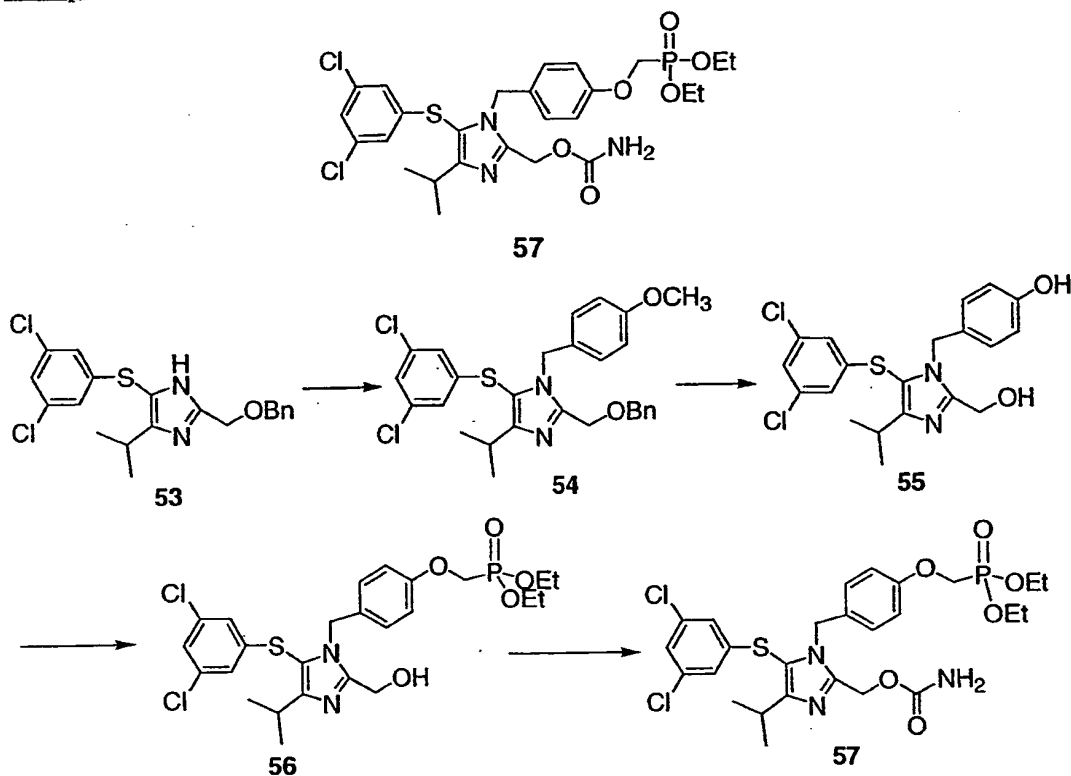
After the reaction mixture was stirred for 1 h at room temperature, the mixture was concentrated under reduced pressure. The residue was purified on silica gel (eluting 4-5% MeOH/CH₂Cl₂) to give the title compound **51** (43 mg, 91%) as a pale yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 8.46 (bs, 2H), 7.31 (m, 2H), 7.20 (d, 2H), 7.14 (m, 1H), 7.05 (s, 1H), 6.81 (bd, 2H), 6.71 (d, 2H), 5.27 (bs, 2H), 5.19 (bs, 2H), 4.07 (m, 2H), 3.98 (m, 1H), 3.63 (m, 1H), 3.18 (m, 3H), 1.83 (m, 2H), 1.80 (m, 2H), 1.58 (m, 2H), 1.35 (m, 2H), 1.32 (d, 6H), 1.30 (d, 1.5H), 1.24 (d, 1.5H), 0.93 (t, 3H). ³¹P NMR (300 MHz, CDCl₃) δ 31.6, 31.3.

Example 25



52

The title compound was prepared following the sequence of steps described in Example 24, except for substituting alanine ethyl ester for alanine *n*-butyl ester. Purification of the crude final product on a preparative TLC plate (5% CH₃OH/CH₂Cl₂) provided 5 mg (75%) of the title compound. ¹H NMR(CDCl₃, 500 MHz): δ 8.46 (d, 2H), 7.32 (d, 2H), 7.20 (d, 2H), 7.15 (s, 1H), 7.05 (s, 1H), 6.82 (d, 2H), 6.70 (s, 2H), 5.27 (s, 2H), 5.19 (s, 2H), 4.12 (m, 2H), 3.70 (t, 2H), 3.19 (m, 2H), 3.12 (t, 2H), 1.48 (m, 3H), 1.47 (t, 3H), 1.25 (d, 6H).

Example 26

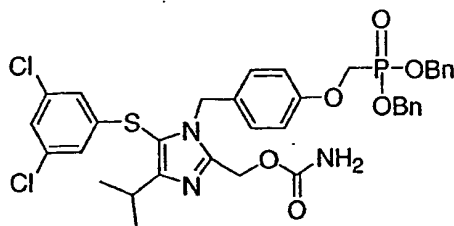
Imidazole 54: A solution of imidazole **53** (267 mg, 0.655 mmol) in THF (10 mL) was treated with 4-methoxybenzyl chloride (0.18 mL, 1.31 mmol), powder NaOH (105 mg, 2.62 mmol), lithium iodide (88 mg, 0.655 mmol), and tetrabutylammonium bromide (105 mg, 0.327 mmol). After stirring for 4 days at room temperature, the resulting mixture was partitioned between EtOAc and sat. NH_4Cl . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 20-40% EtOAc/hexane) to give imidazole **54** (289 mg, 84%) as a colorless oil.

Phenol 55: A solution of benzyl ether **54** (151 mg, 0.286 mmol) in EtOH (5 mL) was treated with conc. HCl (5 mL). After the reaction mixture was warmed to 80°C and stirred for 2 d, the mixture was concentrated under reduced pressure and partitioned between EtOAc and sat. aqueous NaHCO_3 . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 60-70% EtOAc/hexane) to give the alcohol (99 mg, 79%) as a colorless solid. A solution of the alcohol (77 mg, 0.18 mmol) in CH_2Cl_2 (3 mL) was added 1M BBr_3 in CH_2Cl_2 (0.90 mL, 0.90 mmol) at 0°C . After the reaction mixture was stirred for 1 h at 0°C , the mixture was

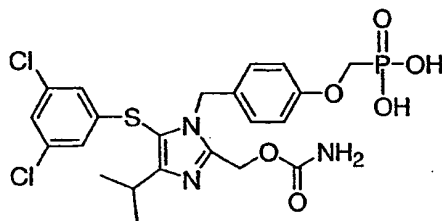
neutralized with sat. NaHCO_3 and extracted with CH_2Cl_2 and EtOAc. The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 4-5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give phenol **55** (68 mg, 89%) as a colorless solid.

Diethylphosphonate 56: To a solution of phenol **55** (21 mg, 0.050 mmol) in CH_3CN (1 mL) and THF (1 mL) was added trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester (18 mg, 0.060 mmol) in CH_3CN (1 mL). After the addition of Cs_2CO_3 (20 mg, 0.060 mmol), the reaction mixture was stirred for 2 h at room temperature. Additional triflate (18 mg, 0.060 mmol) and Cs_2CO_3 (20 mg, 0.060 mmol) were introduced. After the reaction mixture was stirred for another 2 h at room temperature, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and sat. NH_4Cl . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give diethylphosphonate **56** (26 mg, 91%) as a pale yellow oil.

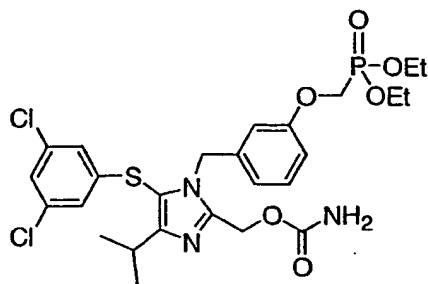
Title compound carbamate 57: A solution of diethylphosphonate **56** (26 mg, 0.045 mmol) in CH_2Cl_2 (2 mL) was treated with trichloroacetyl isocyanate (27 μL , 0.23 mmol). After the reaction mixture was stirred for 10 min at room temperature, the mixture was concentrated under reduced pressure. The residue was transferred to an Al_2O_3 column in 10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$. After soaking on the column for 30 min, the crude product was flushed out with 10% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ and concentrated under reduced pressure. The crude product was purified by preparative thin layer chromatography eluted with 5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$ to give title compound carbamate **57** (22 mg, 79%) as a pale yellow oil. ^1H NMR (500 MHz, CDCl_3) δ 7.00 (s, 1H), 6.88 (d, 2H), 6.76 (d, 2H), 6.62 (s, 2H), 5.24 (s, 2H), 5.18 (s, 2H), 4.26 (q, 4H), 4.21 (d, 2H), 3.15 (m, 1H), 1.38 (t, 6H), 1.29 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 19.1.

Example 27**58**

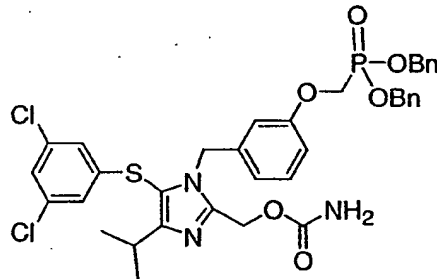
The title compound **58** was prepared following the sequence of steps described in Example 27 with substitution of trifluoro-methanesulfonic acid bis-benzyloxy-phosphorylmethyl ester for trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester. Purification of the crude final product on silica gel eluted with 3-4% MeOH/CH₂Cl₂ provided 33 mg of the title compound. ¹H NMR (500 MHz, CDCl₃) δ 7.37 (m, 10H), 6.96 (s, 1H), 6.85 (d, 2H), 6.70 (d, 2H), 6.62 (s, 2H), 5.23 (s, 2H), 5.17 (s, 2H), 5.13 (m, 4H), 4.18 (d, 2H), 3.16 (m, 1H), 1.30 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.1.

Example 28**59**

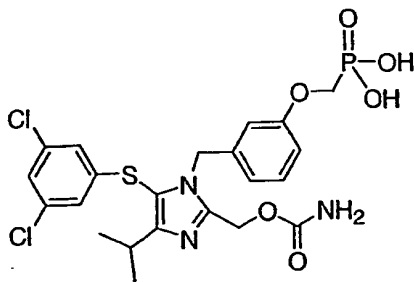
A solution of dibenzylphosphonate **58** (15 mg, 0.020 mmol) was treated 4M HCl in dioxane (1 mL). After the reaction mixture was stirred for 18 h at room temperature, the mixture was concentrated under reduced pressure. The crude product was purified on a C-18 column (eluting 30-40% CH₃CN/H₂O) to give phosphonic acid **59** (8 mg, 71%) as a colorless oil. ¹H NMR (300 MHz, CD₃OD) δ 7.19 (s, 1H), 7.08 (d, 2H), 6.81 (d, 2H), 6.69 (s, 2H), 5.48 (s, 2H), 5.44 (s, 2H), 4.12 (d, 2H), 3.32 (m, 1H), 1.33 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 17.1.

Example 29**60**

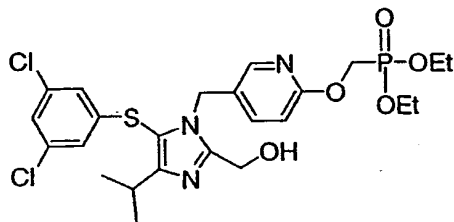
The title compound **60** was prepared following the sequence of steps described in Example 25, except for substituting 3-methoxy benzyl chloride for 4-methoxyl benzyl chloride. Purification of the crude final product on preparative thin layer chromatography eluted with 5% MeOH/CH₂Cl₂ provided 28 mg of the title compound. ¹H NMR (500 MHz, CDCl₃) δ 7.12 (t, 1H), 7.03 (s, 1H), 6.75 (d, 1H), 6.66 (s, 2H), 6.60 (d, 1H), 6.55 (s, 1H), 5.24 (s, 2H), 5.19 (s, 2H), 4.22 (q, 4H), 4.20 (d, 2H), 3.17 (m, 1H), 1.37 (t, 6H), 1.31 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 19.2.

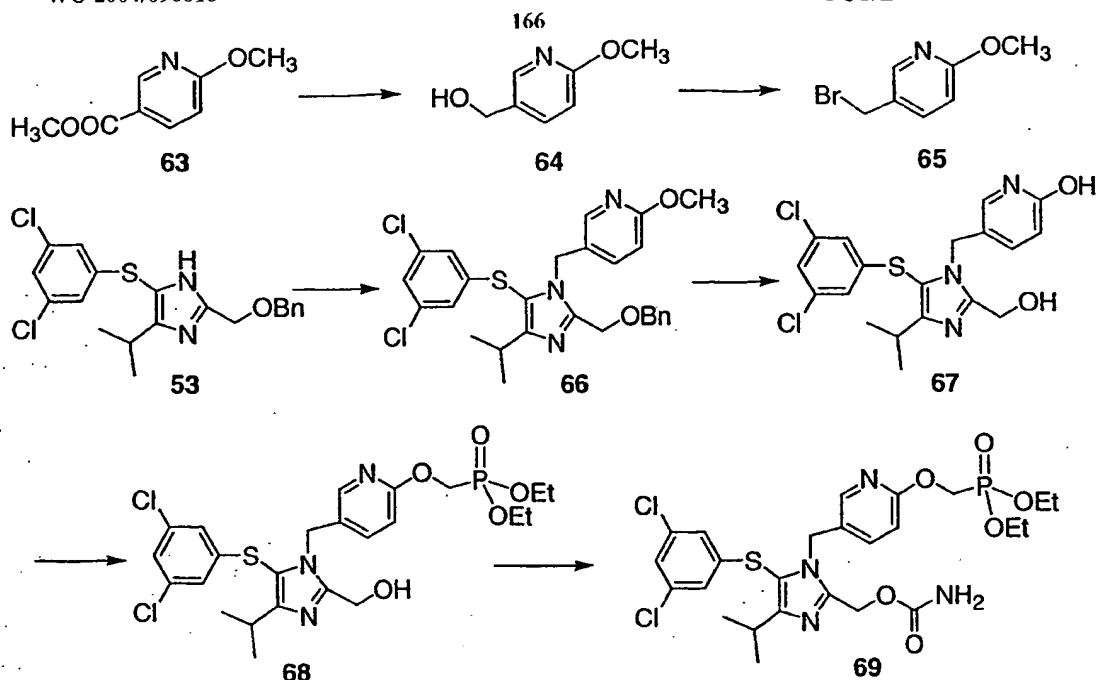
Example 30**61**

The title compound **61** was prepared following the sequence of steps described in Example 26, except for substituting 3-methoxy benzyl chloride for 4-methoxyl benzyl chloride. Purification of the crude final product on silica gel eluted with 3-4% MeOH/CH₂Cl₂ provided 36 mg of the title compound. ¹H NMR (500 MHz, CDCl₃) δ 7.36 (m, 10H), 7.10 (t, 1H), 7.00 (s, 1H), 6.68 (d, 1H), 6.64 (s, 2H), 6.59 (d, 1H), 6.53 (s, 1H), 5.23 (s, 2H), 5.17 (s, 2H), 5.11 (m, 4H), 4.18 (d, 2H), 3.16 (m, 1H), 1.31 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.2.

Example 31**62**

The title compound **62** was prepared following the sequence of steps described in Example 29, except for substituting compound **61** for compound 58. Purification of the crude final product with HPLC (eluting 30-40% CH₃CN/H₂O) provided 7 mg of the title compound. ¹H NMR (300 MHz, CD₃OD) δ 7.18 (s, 1H), 7.13 (t, 1H), 6.81 (d, 1H), 6.77 (s, 2H), 6.72 (s, 1H), 6.68 (d, 1H), 5.49 (s, 2H), 5.37 (s, 2H), 4.12 (d, 2H), 3.33 (m, 1H), 1.34 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 17.0.

Example 32**68**



Alcohol 64: A solution of methyl 6-methoxynicotinate **63** (2.0 g, 12 mmol) in Et₂O (50 mL) was treated with 1.5M DIBAL-H in toluene (16.8 mL, 25.1 mmol) at 0°C. After the reaction mixture was stirred for 1 h at 0°C, the mixture was quenched with 1M sodium potassium tartrate and stirred for an additional 2 h. The aqueous phase was extracted with Et₂O and concentrated to give alcohol **64** (1.54 g, 92%) as a pale yellow oil.

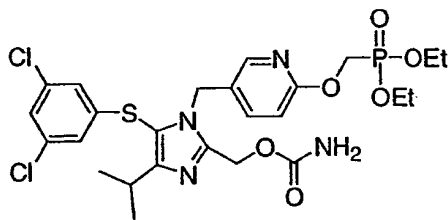
Bromide 65: A solution of alcohol **64** (700 mg, 5.0 mmol) in CH₂Cl₂ (50 mL) was treated with carbon tetrabromide (2.49 g, 7.5 mmol) and triphenylphosphine (1.44 g, 5.5 mmol) at 0°C. After the reaction mixture was stirred for 30 min at room temperature, the mixture was partitioned between CH₂Cl₂ and sat. aqueous NaHCO₃. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 5-10% MeOH/CH₂Cl₂) to give bromide **65** (754 mg, 75%) as colorless crystals.

Imidazole 66: A solution of imidazole **53** (760 mg, 1.86 mmol) and bromide **65** (752 mg, 3.72 mmol) in THF (10 mL) was treated with powder NaOH (298 mg, 7.44 mmol), lithium iodide (249 mg, 1.86 mmol), and tetrabutylammonium bromide (300 mg, 0.93 mmol). After stirring for 14 h at room temperature, the mixture was partitioned between EtOAc and sat. NH₄Cl. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 20-30% EtOAc/hexane) to give imidazole **66** (818 mg, 83%) as a pale yellow oil.

Diol **67**: A solution of benzyl ether **66** (348 mg, 0.658 mmol) in EtOH (3 mL) was treated with conc. HCl (3 mL). After the reaction mixture was warmed to 80°C and stirred for 18 h, the mixture was concentrated under reduced pressure. The crude product was chromatographed on silica gel (eluting 5-10% MeOH/CH₂Cl₂) to give diol **67** (275 mg, 98%) as a colorless solid.

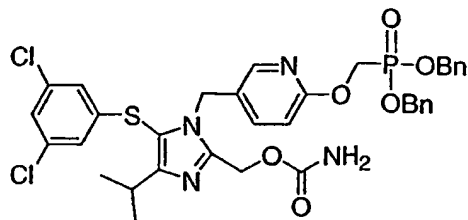
Title compound diethylphosphonate **68**: A solution of diol **67** (40 mg, 0.094 mmol) in THF (1 mL) was treated with trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester (114 mg, 0.38 mmol) in THF (1 mL). After the addition of Ag₂CO₃ (52 mg, 0.19 mmol), the reaction mixture was stirred for 5 d at room temperature. The mixture was quenched with sat. NaHCO₃ and sat. NaCl, and extracted with EtOAc. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed by silica gel (eluting 3-4% MeOH/CH₂Cl₂) and by preparative thin layer chromatography (eluting 4% MeOH/CH₂Cl₂) to give the title compound diethylphosphonate **68** (23 mg, 43%) as a colorless oil. ¹H NMR (300 MHz, CDCl₃) δ 7.92 (s, 1H), 7.39 (d, 1H), 7.00 (s, 1H), 6.65 (d, 1H), 6.55 (d, 2H), 5.20 (s, 2H), 4.81 (s, 2H), 4.55 (d, 2H), 4.21 (m, 4H), 3.08 (m, 1H), 1.35 (t, 6H), 1.20 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.7.

Example 33

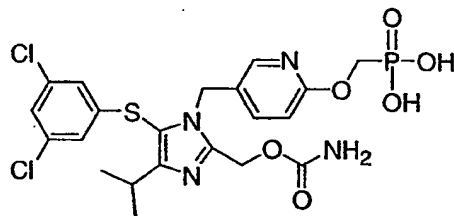


69

A solution of diethylphosphonate **68** (13 mg, 0.023 mmol) in CH₂Cl₂ (0.5 mL) was treated with trichloroacetyl isocyanate (13 µL, 0.11 mmol). After the reaction mixture was stirred for 10 min at room temperature, the mixture was concentrated under reduced pressure. The residue was transferred to an Al₂O₃ column in 10% MeOH/CH₂Cl₂. After soaking on the column for 30 min, the crude product was flushed out with 10% MeOH/CH₂Cl₂ and concentrated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 5% MeOH/CH₂Cl₂) to give carbamate **69** (13 mg, 92%) as a pale yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 7.78 (d, 1H), 7.20 (dd, 1H), 7.03 (t, 1H), 6.65 (d, 1H), 6.62 (d, 2H), 5.24 (s, 2H), 5.16 (s, 2H), 4.74 (bs, 2H), 4.58 (d, 2H), 4.20 (m, 4H), 3.13 (m, 1H), 1.35 (t, 6H), 1.27 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.7.

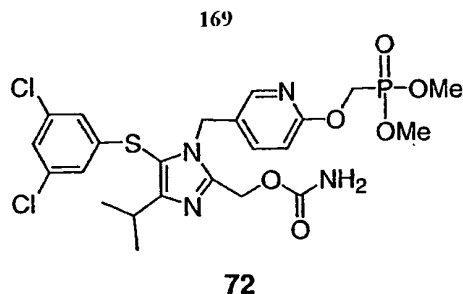
Example 34**70**

The title compound **70** was prepared following the sequence of steps described in Example 32, except for substituting trifluoro-methanesulfonic acid bis-benzyloxy-phosphorylmethyl ester for trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester. Purification of the crude final product on silica gel eluted with 50-60% CH₃CN/H₂O provided 12 mg of the title compound. ¹H NMR (300 MHz; CDCl₃) δ 7.78 (s, 1H), 7.34 (m, 10H), 7.19 (dd, 1H), 7.02 (t, 1H), 6.63 (s, 1H), 6.61 (d, 2H), 5.38 (s, 2H), 5.25 (s, 2H), 5.11 (m, 4H), 4.62 (d, 2H), 3.24 (m, 1H), 1.33 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 21.4.

Example 35**71**

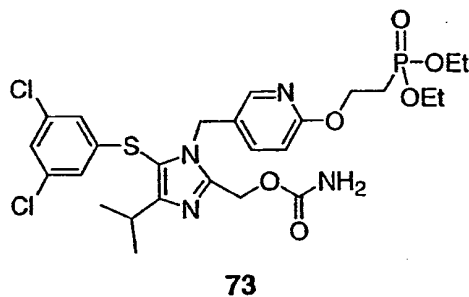
The title compound **71** was prepared following the sequence of steps described in Example 29, except for substituting compound **70** for compound **28**. Purification of the crude final product with HPLC provided 2 mg of the title compound. ¹H NMR (300 MHz, CD₃OD) δ 7.90 (s, 1H), 7.44 (d, 1H), 7.13 (t, 1H), 6.72 (m, 3H), 5.39 (s, 2H), 5.34 (s, 2H), 4.39 (d, 2H), 3.30 (m, 1H), 1.28 (d, 6H).

Example 36



To a solution of phosphonic acid **72** (33 mg, 0.058 mmol) in DMF (2 mL) was added benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (91 mg, 0.175 mmol), $i\text{Pr}_2\text{NEt}$ (30 μL , 0.175 mmol), and MeOH (0.24 mL, 5.83 mmol). After the reaction mixture was stirred for 2 d at room temperature, the mixture was partitioned between EtOAc and sat. NH_4Cl . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. Purification of the crude final product on silica gel eluted with 3-5% MeOH/ CH_2Cl_2 and by preparative thin layer chromatography (eluting 5% MeOH/ CH_2Cl_2) provided 6 mg of the title compound as a colorless solid. ^1H NMR (300 MHz, CDCl_3) δ 7.79 (d, 1H), 7.21 (dd, 1H), 7.04 (s, 1H), 6.66 (d, 1H), 6.62 (d, 2H), 5.25 (s, 2H), 5.17 (s, 2H), 4.70 (bs, 2H), 4.63 (d, 2H), 3.84 (d, 6H), 3.14 (m, 1H), 1.28 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 23.2.

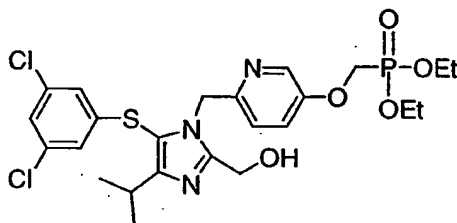
Example 37



A solution of diol **67** (50 mg, 0.118 mmol) in CH_2Cl_2 (5 mL) was treated with diethyl (2-bromoethyl)-phosphonate (64 μL , 0.354 mmol) and Ag_2CO_3 (65 mg, 0.236 mmol). After the reaction mixture was stirred for 3 d at 40°C , additional phosphonate (64 μL , 0.354 mmol), Ag_2CO_3 (65 mg, 0.236 mmol), and benzene (5 mL) were introduced. After the reaction mixture was stirred for another 4 days at 70°C , the mixture was filtered through a medium-fritted funnel. The crude product was chromatographed by silica gel (eluting 4-5% MeOH/ CH_2Cl_2) to give diethylphosphonate **74** (8 mg, 12%) as a colorless oil. ^1H NMR (300

MHz, CDCl_3) δ 7.81 (bs, 1H), 7.17 (dd, 1H), 7.03 (t, 1H), 6.60 (d, 2H), 6.52 (d, 2H), 5.25 (s, 2H), 5.15 (s, 2H), 4.71 (bs, 2H), 4.47 (m, 2H), 4.14 (m, 4H), 3.12 (m, 1H), 2.27 (m, 2H), 1.34 (t, 6H), 1.27 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 28.0.

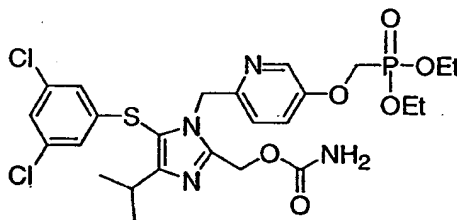
Example 38



74

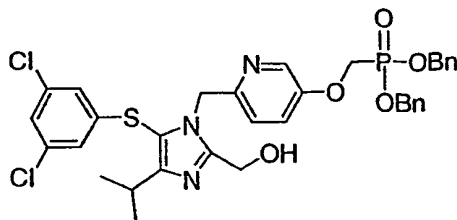
The title compound **74** was prepared following the sequence of steps described in Example 33, except for substituting 6-bromomethyl-3-methoxy pyridine for 5-bromomethyl-2-methoxy pyridine **65**. Purification of the crude final product on silica gel with 4-5% MeOH/ CH_2Cl_2 provided 66 mg of the title compound. ^1H NMR (300 MHz, CDCl_3) δ 8.17 (d, 1H), 7.01 (d, 1H), 6.93 (m, 2H), 6.41 (d, 2H), 5.26 (s, 2H), 4.94 (s, 2H), 4.22 (q, 4H), 4.12 (m, 2H), 3.08 (m, 1H), 1.38 (t, 6H), 1.25 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 17.7.

Example 39

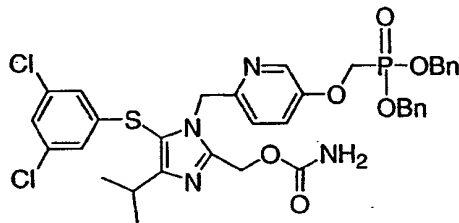


75

The title compound **75** was prepared following the sequence of steps described in Example 34, except for substituting compound **74** for compound **33**. Purification of the crude final product on preparative thin layer chromatography eluted with 5% MeOH/ CH_2Cl_2 provided 15 mg the title compound. ^1H NMR (500 MHz, CDCl_3) δ 8.18 (d, 1H), 6.98 (m, 1H), 6.96 (m, 1H), 6.79 (d, 1H), 6.58 (d, 2H), 5.35 (s, 2H), 5.32 (s, 2H), 4.83 (bs, 2H), 4.25 (q, 4H), 4.24 (m, 2H), 3.14 (m, 1H), 1.39 (t, 6H), 1.28 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 18.1.

Example 40**76**

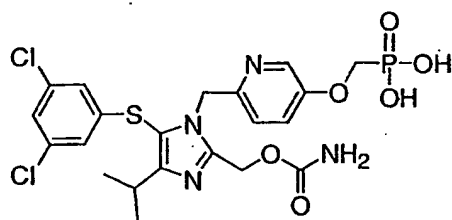
The title compound **76** was prepared following the sequence of steps described in Example 39, except for substituting trifluoro-methanesulfonic acid bis-benzyloxy-phosphorylmethyl ester for trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester. Purification of the crude final product on silica gel eluted with 4% MeOH/CH₂Cl₂ provided 67 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 8.05 (d, 1H), 7.36 (m, 10H), 6.95 (d, 1H), 6.81 (m, 2H), 6.37 (d, 2H), 5.22 (s, 2H), 5.13 (m, 4H), 4.91 (s, 2H), 4.11 (d, 2H), 3.05 (m, 1H), 1.22 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 18.8.

Example 41**77**

The title compound **77** was prepared following the sequence of steps described in Example 34, except for substituting compound **76** for compound **33**. Purification of the crude final product on silica gel eluted with 4-5% MeOH/CH₂Cl₂ provided 35 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 8.07 (d, 1H), 7.36 (m, 10H), 6.85 (m, 2H), 6.72 (d, 1H), 6.55 (d, 2H), 5.35 (s, 2H), 5.29 (s, 2H), 5.13 (m, 4H), 4.74 (bs, 2H), 4.15 (d, 2H), 3.13 (m, 1H), 1.28 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 19.2.

Example 42

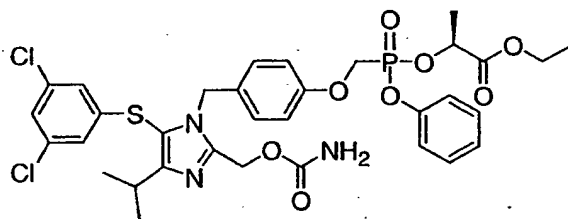
172



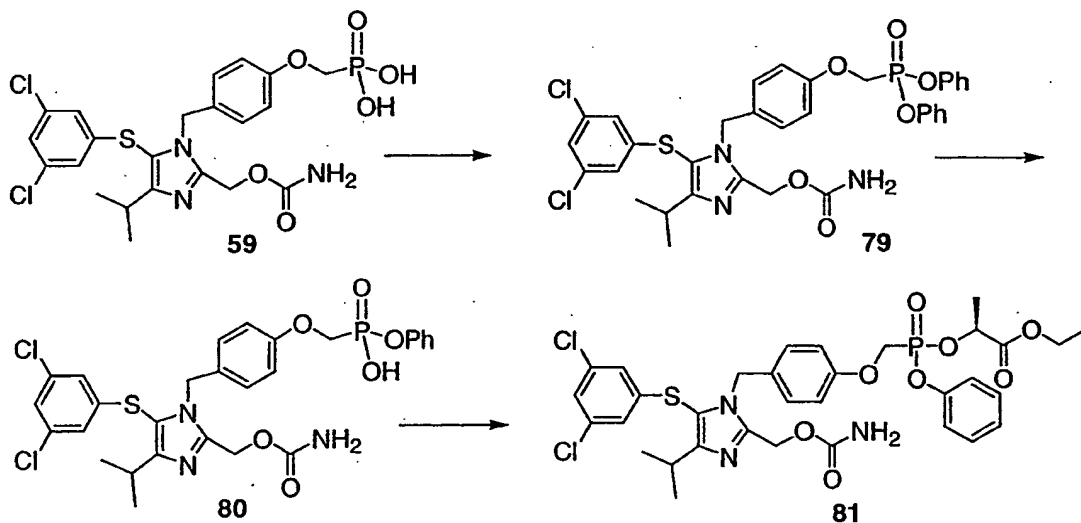
78

The title compound **78** was prepared following the sequence of steps described in Example 29, except for substituting compound **77** for compound **28**. Purification of the crude final product on a C-18 column eluted with 30% CH₃CN/H₂O provided 6 mg of the title compound. ¹H NMR (300 MHz, CD₃OD) δ 8.16 (bs, 1H), 7.21 (bs, 2H), 7.18 (bs, 1H), 6.70 (d, 2H), 5.64 (s, 2H), 5.49 (s, 2H), 4.21 (d, 2H), 3.34 (m, 1H), 1.34 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 16.0.

Example 43



81



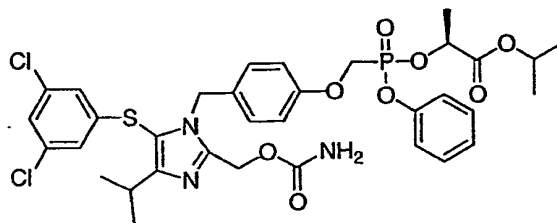
Diphenylphosphonate **79**: A solution of phosphonic acid **59** (389 mg, 0.694 mmol) in pyridine (5 mL) was treated with phenol (653 mg, 6.94 mmol) and 1,3-

dicyclohexylcarbodiimide (573 mg, 2.78 mmol). After stirring at 70°C for 2 h, the mixture was diluted with CH₃CN and filtered through a fritted funnel. The filtrate was partitioned between EtOAc and sat. NH₄Cl, and extracted with EtOAc. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 60-80% EtOAc/hexane) to give diphenylphosphonate **79** (278 mg, 56%) as a colorless oil.

Phosphonic acid 80: A solution of diphenylphosphonate **79** (258 mg, 0.362 mmol) in CH₃CN (20 mL) was treated with 1N NaOH (0.72 mL, 0.724 mmol) at 0°C. After the reaction mixture was stirred for 3 h at 0°C, the mixture was filtered through Dowex 50WX8-400 acidic resin (380 mg), rinsed with MeOH, and concentrated under reduced pressure to give phosphonic acid **80** (157 mg, 68%) as a colorless solid.

Title compound 81: A solution of phosphonic acid **80** (35 mg, 0.055 mmol) in CH₃CN (1 mL) and THF (1 mL) was treated with thionyl chloride (12 µL, 0.16 mmol). After the reaction mixture was warmed to 70°C and stirred for 2 h, the mixture was concentrated under reduced pressure. The residue was then dissolved in CH₂Cl₂ (2 mL) and cooled to 0°C. Triethylamine (31 µL, 0.22 mmol) and ethyl S-(-)-lactate (19 µL, 0.16 mmol) were added. After stirring for 1 h at 0°C and 1 h at room temperature, the reaction mixture was neutralized with sat. NH₄Cl and extracted with CH₂Cl₂ and EtOAc. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 70% EtOAc/hexane) to give ethyl lactate **81** (7 mg, 17%) as a colorless solid. ¹H NMR (300 MHz, CDCl₃) δ 7.30 (m, 5H), 6.99 (d, 1H), 6.82 (m, 4H), 6.63 (d, 2H), 5.23 (s, 2H), 5.18 (s, 2H), 5.14 (m, 1H), 4.67 (bs, 2H), 4.51 (d, 2H), 4.20 (m, 2H), 3.16 (m, 1H), 1.61 (d, 1.5H), 1.50 (d, 1.5H), 1.30 (d, 6H), 1.24 (m, 3H). ³¹P NMR (300 MHz, CDCl₃) δ 17.0, 15.0.

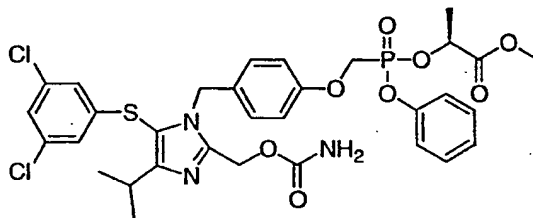
Example 44



82

The title compound **82** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with isopropyl lactate. Purification of the crude final product on silica gel eluted with 70-90% EtOAc/hexane provided 5.4 mg of the title compound. ^1H NMR (300 MHz, CDCl_3) δ 7.35 (m, 3H), 7.25 (m, 3H), 7.0 (s, 0.5H), 6.98 (s, 0.5H), 6.86 (m, 2H), 6.79 (m, 2H), 6.64 (s, 1H), 6.61 (s, 1H), 5.22 (s, 2H), 5.17 (s, 2H), 5.06 (b, 1H), 4.62 (b, 2H), 4.53 (m, 2H), 4.38 (q, 1H), 3.15 (m, 1H), 1.60 (d, 1.5H), 1.48 (d, 1.5H), 1.30 (d, 3H), 1.28 (d, 3H), 1.20 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 17.04, 14.94 (1:1 diastereomeric ratio).

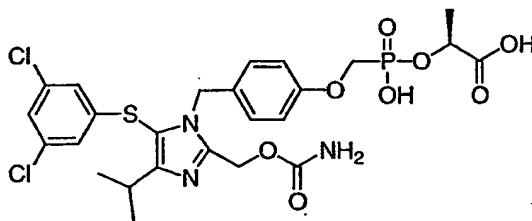
Example 45



83

The title compound **83** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with methyl lactate. Purification of the crude final product on silica gel eluted with 70-90% EtOAc/hexane provided 2.7 mg of the title compound. ^1H NMR (300 MHz, CD_3CN) δ 7.40 (m, 2H), 7.25 (m, 3H), 7.08 (s, 1H), 6.98 (d, 2H), 6.77 (d, 2H), 6.64 (s, 2H), 5.20 (s, 2H), 5.16 (s, 2H), 5.13 (b, 1H), 4.47 (m, 2H), 3.72 (s, 2H), 3.67 (s, 1H), 3.09 (m, 1H), 1.56 (d, 1H), 1.51 (d, 2H), 1.20 (d, 6H). ^{31}P NMR (300 MHz, CD_3CN) δ 16.86, 15.80 (2.37:1 diastereomeric ratio).

Example 46

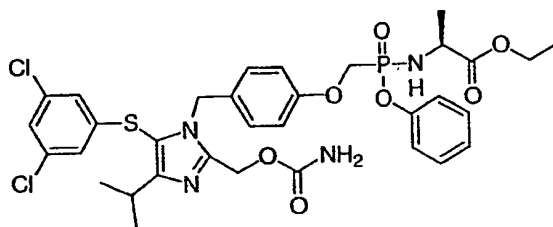


84

A solution of mono-lactate phosphonate compound **83** (131 mg, 0.18 mmol) in DMSO/MeCN (1 mL/2 mL) and PBS buffer (10 mL) was treated with esterase (400 μL). After the reaction mixture was warmed to 40°C and stirred for 7 days, the mixture was filtered

and concentrated under reduced pressure. Purification of the crude product on C₁₈ column eluted with MeCN/H₂O provided 17.3 mg (15 %) of the title compound **84**. ¹H NMR (300 MHz, CD₃OD) δ 7.20 (s, 1H), 7.02 (d, 2H), 6.79 (d, 2H), 6.71 (s, 2H), 5.40 (s, 2H), 5.35 (s, 2H), 5.34 (b, 1H) 4.10 (bd, 2H), 3.26 (m, 1H), 1.50 (d, 3H), 1.30 (d, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 14.2.

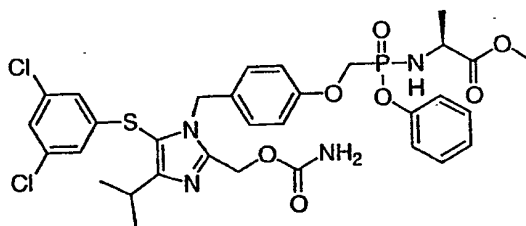
Example 47



85

The title compound **85** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with L-alanine ethyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 80% EtOAc/hexane provided 7 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 7.26 (m, 5H), 6.98 (d, 1H), 6.87 (d, 2H), 6.73 (t, 2H), 6.62 (s, 2H), 5.21 (s, 2H), 5.17 (s, 2H), 4.28 (bs, 2H), 4.25 (m, 2H), 4.10 (m, 2H), 4.02 (m, 1H), 3.66 (m, 1H), 3.14 (m, 1H), 1.28 (d, 6H), 1.24 (m, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.2, 19.1.

Example 48

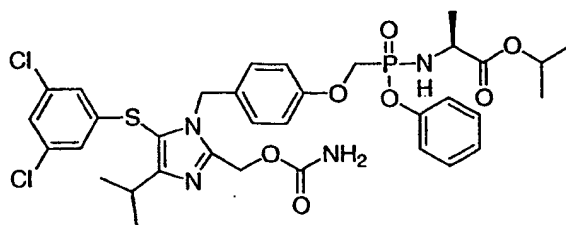


86

The title compound **86** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with L-alanine methyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 80% EtOAc/hexane provided 8 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 7.25 (m, 5H), 6.98 (d, 1H), 6.88 (d, 2H), 6.73 (t, 2H), 6.61 (bs, 2H), 5.21 (d, 2H), 5.17 (s, 2H),

4.66 (bs, 2H), 4.25 (m, 3H), 3.66 (s, 1.5H), 3.64 (m, 1H), 3.59 (m, 1.5H), 3.14 (m, 1H), 1.36 (t, 6H), 1.28 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 20.2, 19.0.

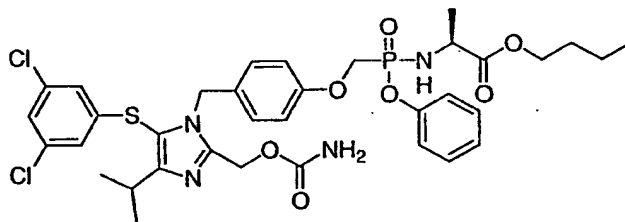
Example 49



87

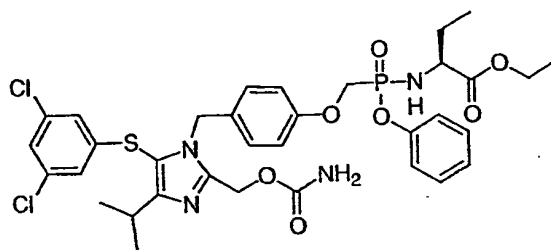
The title compound **87** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with L-alanine isopropyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 80% EtOAc/hexane provided 7 mg of the title compound. ^1H NMR (300 MHz, CDCl_3) δ 7.25 (m, 5H), 6.98 (m, 1H), 6.87 (d, 2H), 6.74 (m, 2H), 6.61 (bs, 2H), 5.22 (d, 2H), 5.18 (s, 2H), 4.93 (m, 1H), 4.68 (bs, 2H), 4.25 (m, 3H), 3.66 (s, 1H), 3.15 (m, 1H), 1.34 (m, 3H), 1.29 (d, 6H), 1.17 (m, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 20.1, 19.1.

Example 50

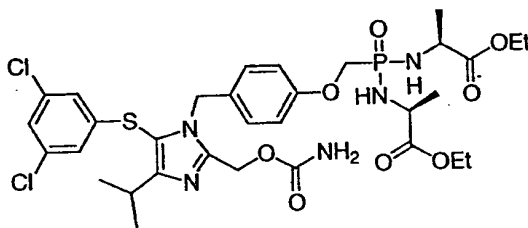


88

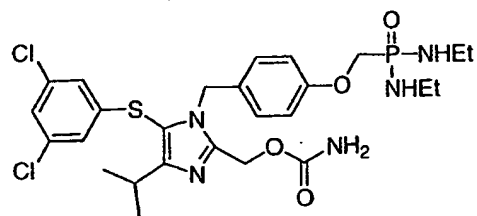
The title compound **88** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with L-alanine *n*-butyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 80% EtOAc/hexane provided 6 mg of the title compound. ^1H NMR (300 MHz, CDCl_3) δ 7.25 (m, 5H), 6.98 (bd, 1H), 6.88 (d, 2H), 6.73 (t, 2H), 6.61 (d, 2H), 5.22 (d, 2H), 5.17 (s, 2H), 4.63 (bs, 2H), 4.25 (m, 3H), 4.06 (m, 2H), 3.65 (m, 1H), 3.14 (m, 1H), 1.58 (m, 4H), 1.36 (m, 3H), 1.28 (d, 6H), 0.90 (t, 3H). ^{31}P NMR (300 MHz, CDCl_3) δ 20.2, 19.1.

Example 51**89**

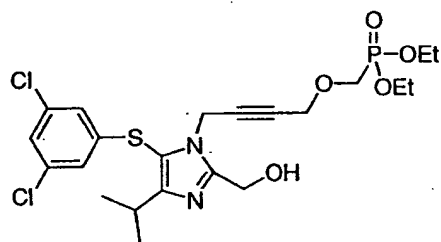
The title compound **89** was prepared following the sequence of steps described in Example 44, except for reacting monophosphonic acid **80** with L-alanine *n*-butyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 80% EtOAc/hexane provided 4 mg of the title compound. ^1H NMR (300 MHz, CDCl_3) δ 7.24 (m, 5H), 6.98 (m, 1H), 6.87 (d, 2H), 6.74 (t, 2H), 6.62 (d, 2H), 5.21 (d, 2H), 5.17 (s, 2H), 4.64 (bs, 2H), 4.24 (m, 2H), 4.11 (m, 3H), 3.58 (m, 1H), 3.15 (m, 1H), 1.28 (d, 6H), 1.19 (m, 5H), 0.84 (m, 3H). ^{31}P NMR (300 MHz, CDCl_3) δ 20.4, 19.4.

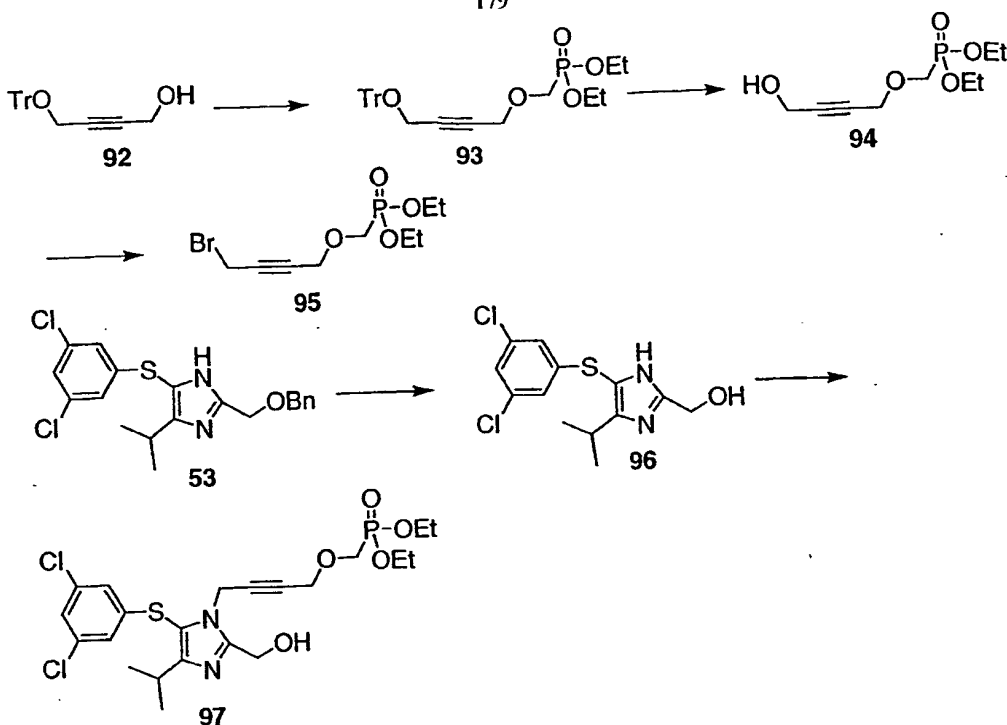
Example 52**90**

To a solution of phosphonic acid **59** (61 mg, 0.11 mmol) in DMF (1 mL) was added benzotriazol-1-yloxytripyrrolidino-phosphonium hexafluorophosphate (169 mg, 0.32 mmol), L-alanine ethyl ester (50 mg, 0.32 mmol), and DIEA (151 μL , 0.87 mmol). The reaction mixture was stirred for 5 hours at room temperature. Then the mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with HCl (5 % aq), and extracted with EtOAc (3x). The organic phase was washed with sat. NaHCO_3 , dried over Na_2SO_4 , and evaporated under reduced pressure. The crude product was purified on silica gel eluted with 5-8% MeOH/ CH_2Cl_2 to give 5.5 mg of compound bis-amidate **90** as white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.06 (s, 1H), 6.88 (d, 2H), 6.73 (d, 2H), 6.62 (s, 2H), 5.23 (s, 2H), 5.17 (s, 2H), 4.70 (bs, 2H), 4.25 (bm, 8H), 3.40 (q, 2H), 3.16 (m, 1H), 1.44 (t, 6H), 1.24 (d, 6H). ^{31}P NMR (300 MHz, CDCl_3) δ 19.41.

Example 53**91**

The title compound **91** was prepared following the sequence of steps described in Example 52, except for substituting ethyl amine for L-alanine ethyl ester. Purification of the crude final product on silica gel eluted with 4-10% MeOH/CH₂Cl₂ provided 14.8 mg of the title compound. ¹H NMR (300 MHz, CD₃OD) δ 7.07 (s, 1H), 6.99 (d, 2H), 6.77 (d, 2H), 6.60 (s, 2H), 5.27 (s, 2H), 5.22 (s, 2H), 4.07 (d, 2H), 3.09 (m, 1H), 3.01 (bm, 4H), 1.24 (d, 6H), 1.16 (t, 6H). ³¹P NMR (300 MHz, CD₃OD) δ 24.66.

Example 54**97**



Diethylphosphonate 93: A solution of alcohol 92 (200 mg, 0.609 mmol) in THF (5 mL) was treated with 60% NaH in mineral oil (37 mg, 0.914 mmol) at 0°C. After the reaction mixture was stirred for 5 min at 0°C, trifluoro-methanesulfonic acid diethoxyphosphorylmethyl ester (219 mg, 0.731 mmol) was added in THF (3 mL). After the reaction mixture was stirred for an additional 30 min, the mixture was quenched with sat. NH_4Cl and extracted with EtOAc. The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure to give crude diethylphosphonate 93 as a colorless oil.

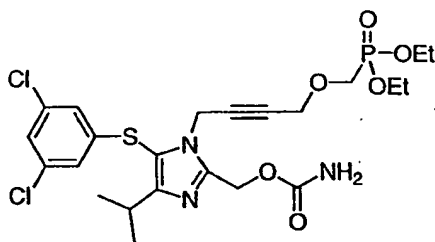
Alcohol 94: A solution of diethylphosphonate 93 (291 mg, 0.609 mmol) in CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (0.5 mL). After the reaction mixture was stirred for 30 min at room temperature, the mixture was concentrated under reduced pressure. The crude product was purified on silica gel (eluting 4-5% MeOH/ CH_2Cl_2) to give alcohol 94 (135 mg, 94% over 2 steps) as a colorless oil.

Bromide 95: A solution of alcohol 94 (134 mg, 0.567 mmol) in CH_2Cl_2 (5 mL) was treated with carbon tetrabromide (282 mg, 0.851 mmol) and triphenylphosphine (164 mg, 0.624 mmol). After stirring at room temperature for 1 h, the mixture was partitioned between CH_2Cl_2 and sat. NaHCO_3 . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified twice on silica gel (eluting 60-100% EtOAc/hexane, followed by eluting 0-2% MeOH/ CH_2Cl_2) to give bromide 95 (80 mg, 47%) as a colorless oil.

Imidazole 96: A solution of benzyl ether **53** (2.58 g, 6.34 mmol) in EtOH (60 mL) was treated with conc. HCl (60 mL). After the reaction mixture was warmed to 100°C and stirred for 18 h, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and sat. NaHCO₃. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 8-9% MeOH/CH₂Cl₂) to give imidazole **96** (1.86 g, 93%) as a colorless solid.

Title compound 97: A solution of imidazole **96** (54 mg, 0.170 mmol) and bromide **95** (56 mg, 0.187 mmol) in THF (3 mL) was treated with powder NaOH (14 mg, 0.340 mmol), lithium iodide (23 mg, 0.170 mmol), and tetrabutylammonium bromide (27 mg, 0.085 mmol) were then added. After stirring at room temperature for 2 h, the mixture was partitioned between EtOAc and sat. NH₄Cl. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 3-4% MeOH/CH₂Cl₂) and by preparative thin layer chromatography (eluting 5% MeOH/CH₂Cl₂) to give alcohol **97** (42 mg, 46%) as a pale yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 7.13 (bs, 1H), 6.86 (d, 2H), 4.92 (s, 2H), 4.87 (s, 2H), 4.16 (m, 6H), 3.73 (d, 2H), 3.10 (m, 1H), 1.34 (t, 6H), 1.21 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.8.

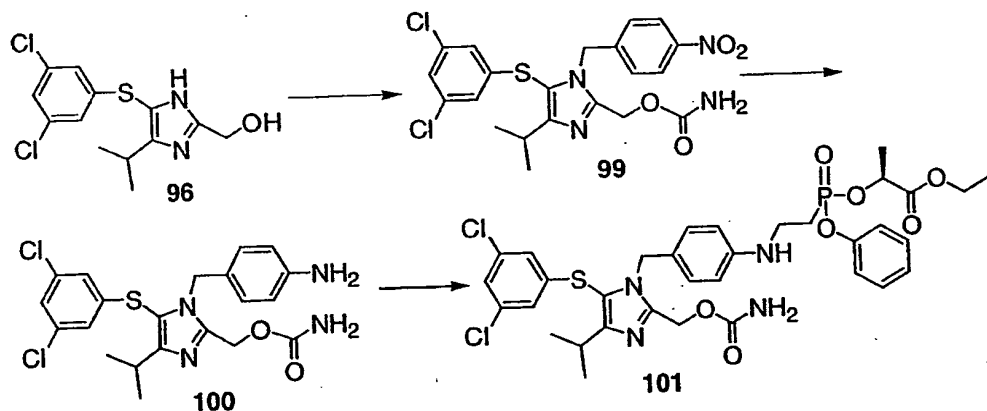
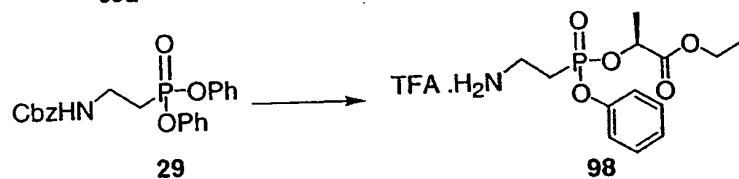
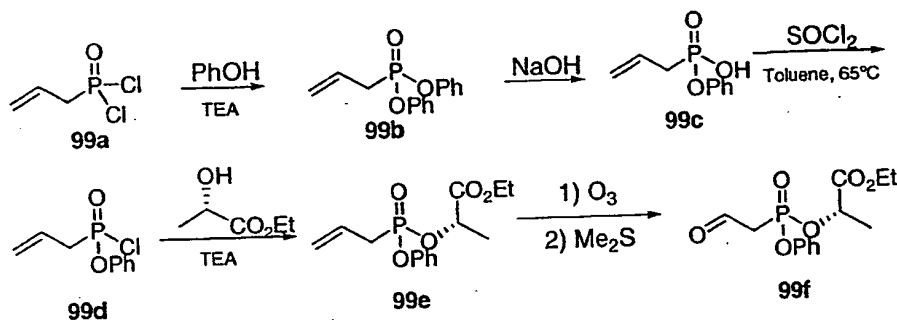
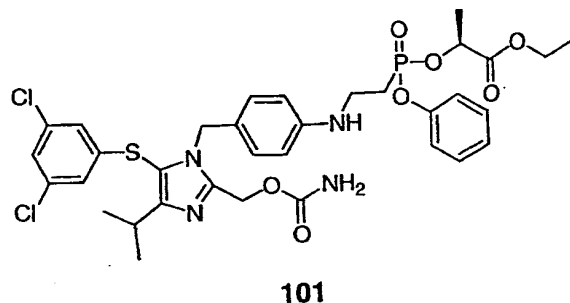
Example 55



97a

The title compound **97a** was prepared following the sequence of steps described in Example 32 by substituting compound **97a** for compound **68**. Purification of the crude final product on silica gel eluted with 3-4% MeOH/CH₂Cl₂ provided 13 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 7.13 (t, 1H), 6.87 (d, 2H), 5.29 (s, 2H), 4.87 (s, 2H), 4.14 (m, 6H), 3.72 (d, 2H), 3.13 (m, 1H), 1.33 (t, 6H), 1.26 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 21.2.

Example 56



Monophenol Allylphosphonate **99c**: To a solution of allylphosphonic dichloride **99a** (4 g, 25.4 mmol) and phenol (5.2 g, 55.3 mmol) in CH_2Cl_2 (40 mL) at 0°C was added TEA (8.4 mL, 60 mmol). After stirred at room temperature for 1.5 h, the mixture was diluted with hexane-ethyl acetate and washed with HCl (0.3 N) and water. The organic phase was dried over MgSO_4 , filtered and concentrated under reduced pressure. The residue was filtered through a pad of silica gel (eluted with 2:1 hexane-ethyl acetate) to afford crude product diphenol allylphosphonate **99b** (7.8 g, containing the excessive phenol) as an oil which was used directly without any further purification. The crude material was dissolved in CH_3CN

(60 mL), and NaOH (4.4N, 15 mL) was added at 0°C. The resulted mixture was stirred at room temperature for 3 h, then neutralized with acetic acid to pH = 8 and concentrated under reduced pressure to remove most of the acetonitrile. The residue was dissolved in water (50 mL) and washed with CH₂Cl₂ (3X25 mL). The aqueous phase was acidified with concentrated HCl at 0°C and extracted with ethyl acetate. The organic phase was dried over MgSO₄, filtered, evaporated and co-evaporated with toluene under reduced pressure to yield desired monophenol allylphosphonate **99c** (4.75 g, 95%) as an oil.

Monolactate Allylphosphonate 99e: A solution of monophenol allylphosphonate **99c** (4.75 g, 24 mmol) in toluene (30 mL) was treated with SOCl₂ (5 mL, 68 mmol) and DMF (0.05 mL). After stirred at 65°C for 4 h, the reaction was completed as shown by ³¹P NMR. The reaction mixture was evaporated and co-evaporated with toluene under reduced pressure to give mono chloride **99d** (5.5 g) as an oil. A solution of chloride **99d** in CH₂Cl₂ (25 mL) at 0°C was added ethyl (s)-lactate (3.3 mL, 28.8 mmol), followed by TEA. The mixture was stirred at 0°C for 5 min then at room temperature for 1 h, and concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N), the organic phase was washed with water, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired monolactate **99e** (5.75 g, 80%) as an oil (2:1 mixture of two isomers).

Aldehyde 99f: A solution of allylphosphonate **99e** (2.5 g, 8.38 mmol) in CH₂Cl₂ (30 mL) was bubbled with ozone air at -78°C until the solution became blue, then bubbled with nitrogen until the blue color disappeared. Methyl sulfide (3 mL) was added at -78°C. The mixture was warmed up to room temperature, stirred for 16 h and concentrated under reduced pressure to give desired aldehyde **99f** (3.2 g, as a 1:1 mixture of DMSO).

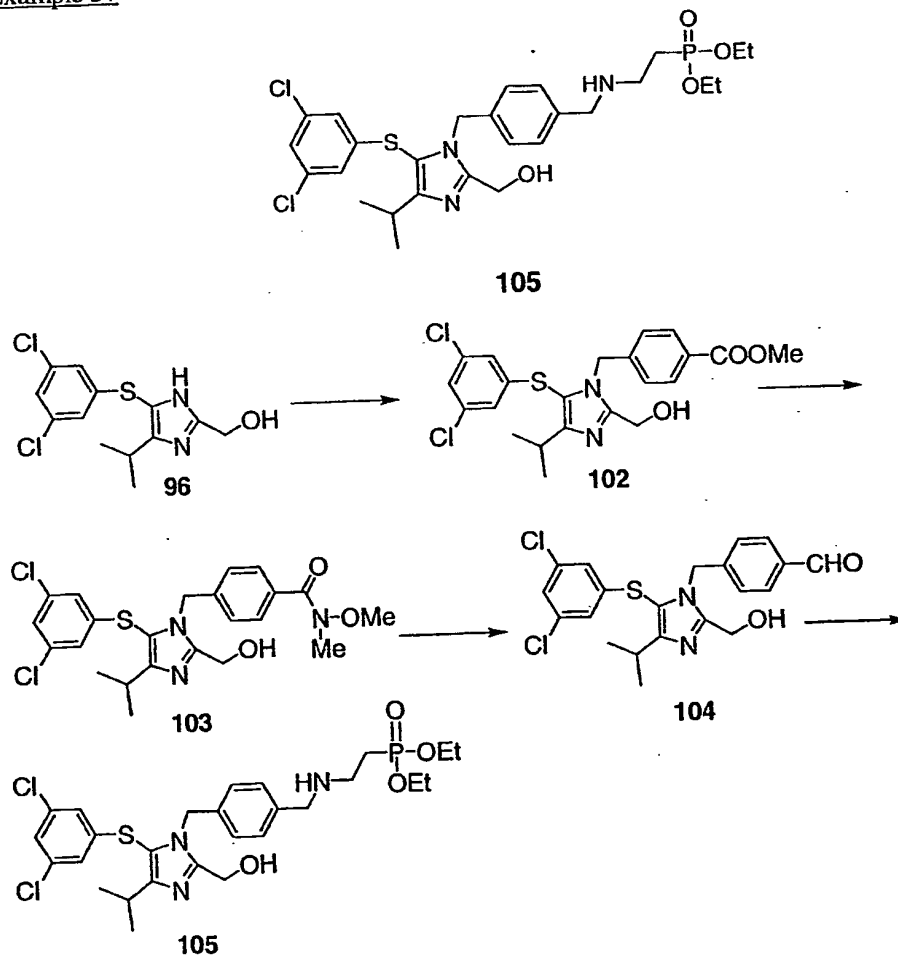
Compound **98** was prepared from compound **29** following the sequence of steps described in Example 22. Compound **99** was prepared from compound **96** following the sequence of steps described in Example 54 and 55, except for substituting 4-nitro benzyl bromide for compound **95**.

Aniline 100: To a solution of compound **99** (100 mg, 0.202 mmol) in EtOH (2 mL) was added acetic acid (2 mL) and zinc dust (40 mg, 0.606 mmol). After the reaction mixture was stirred for 30 min at room temperature, the mixture was concentrated under reduced pressure. The crude product was purified on silica gel (eluting 5-6% MeOH/CH₂Cl₂) to give aniline **100** (43 mg, 41%) as a yellow oil.

Title compound phosphonate 101: To a solution of aniline **100** (22 mg, 0.042 mmol) and aldehyde **99f** (17 mg, 0.046 mmol) in MeOH (2 mL) was added acetic acid (10 µL, 0.17

mmol) and 4Å molecular sieves (10 mg). After the reaction mixture was stirred for 2 h at room temperature, NaCNBH_3 (5 mg, 0.084 mmol) was added. After the reaction mixture was stirred for an additional 4 h at room temperature, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and sat. NaHCO_3 . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluting 5-6% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give title compound phosphonate **101** (25 mg, 79%) as a colorless oil. ^1H NMR (500 MHz, CDCl_3) δ 7.34 (dd, 2H), 7.21 (m, 3H), 7.02 (bs, 1H), 6.79 (d, 2H), 6.64 (t, 2H), 6.42 (dd, 2H), 5.21 (s, 2H), 5.10 (s, 2H), 5.02 (m, 1H), 4.75 (bs, 2H), 4.20 (m, 2H), 3.53 (m, 2H), 3.13 (m, 1H), 2.31 (m, 2H), 1.58 (d, 1.5H), 1.38 (d, 1.5H), 1.28 (d, 6H), 1.25 (t, 3H). ^{31}P NMR (300 MHz, CDCl_3) δ 28.4, 26.5.

Example 57



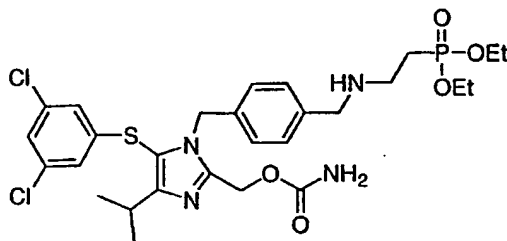
Compound **102** was prepared from compound **96** following the sequence of steps described in Example 54, except for substituting methyl 4-bromomethyl benzoate for compound **95**.

Amide 103: A solution of ester **102** (262 mg, 0.563 mmol) in THF (5 mL) and CH₃CN (2 mL) was treated with 1N NaOH (1.13 mL, 1.13 mmol). After the reaction mixture was stirred for 2 h at 60°C, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and 1N HCl. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 5-10% MeOH/CH₂Cl₂) to give the carboxylic acid (120 mg, 47%) as a colorless oil. A solution of the above carboxylic acid (120 mg, 0.266 mmol) and N,O-dimethylhydroxylamine (29 mg, 0.293 mmol) in DMF (3 mL) was treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (61 mg, 0.319 mmol), 1-hydroxybenzotriazole hydrate (43 mg, 0.319 mmol), and triethylamine (55 µL, 0.399 mmol). After the reaction mixture was stirred for 18 h at room temperature, the mixture was partitioned between EtOAc and H₂O. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 3-4% MeOH/CH₂Cl₂) to give the amide **103** (107 mg, 81%) as a colorless oil.

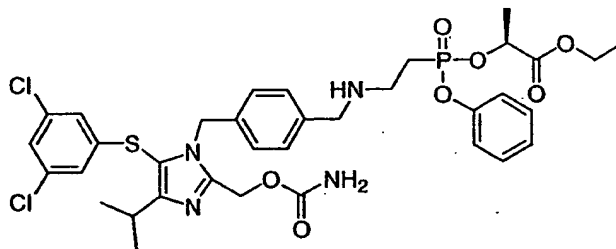
Aldehyde 104: A solution of amide **103** (106 mg, 0.214 mmol) in THF (5 mL) was treated with 1.5M DIBAL-H in toluene (0.43 mL, 0.642 mmol) at 0°C. After the reaction mixture was stirred for 1 h at 0°C, the mixture was quenched with 1M sodium potassium tartrate and stirred for an additional 3 d. The aqueous phase was extracted with EtOAc, and the organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure to give crude aldehyde **104** as a colorless oil.

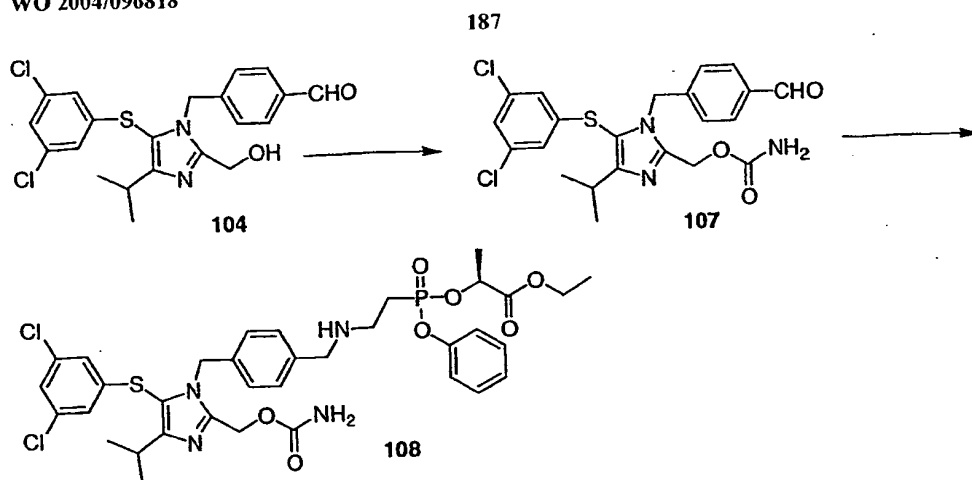
Title compound 105: To a solution of aldehyde **104** (91 mg, 0.21 mmol) in MeOH (5 mL) was added diethyl(aminoethyl) phosphonate (63 mg, 0.231 mmol), acetic acid (48 µL, 0.231 mmol) and 4Å molecular sieves (10 mg). After the reaction mixture was stirred for 2 h at room temperature, NaCNBH₃ (26 mg, 0.42 mmol) was added. After the reaction mixture was stirred for an additional 18 h at room temperature, the mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc and sat. NaHCO₃. The organic phase was dried over Na₂SO₄, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 5-10% MeOH/CH₂Cl₂) to give phosphonate **105** (10 mg, 8% over 2 steps) as a colorless oil. ¹H NMR (300 MHz, CD₃OD) δ 7.15 (d, 2H), 7.10 (t, 1H), 7.06 (d, 2H), 6.65 (t, 2H), 5.34 (s, 2H), 4.73 (s, 2H), 4.09 (m, 4H).

3.68 (s, 2H), 3.12 (m, 1H), 2.83 (m, 2H), 2.04 (m, 2H), 1.30 (t, 6H), 1.24 (d, 6H). ^{31}P NMR (300 MHz, CD_3OD) δ 30.6.

Example 58**106**

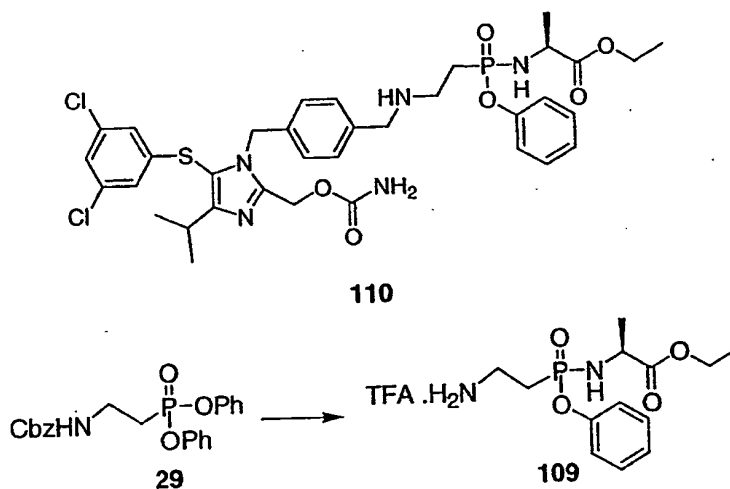
The title compound **106** was prepared following the sequence of steps described in Example 34, except for substituting compound **105** for compound **68**. Purification of the crude final product on preparative thin layer chromatography eluted with 7% MeOH/CH₂Cl₂ provided 6 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) δ 7.15 (d, 2H), 7.02 (bs, 1H), 6.88 (d, 2H), 6.67 (t, 2H), 5.21 (s, 2H), 5.17 (s, 2H), 4.76 (bs, 2H), 4.08 (m, 4H), 3.70 (s, 2H), 3.15 (m, 1H), 2.86 (m, 2H), 1.97 (m, 2H), 1.31 (t, 6H), 1.29 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 30.6.

Example 59**108**



Compound **107** was prepared following the sequence of steps described in Example 34, except for substituting compound **104** for compound **68**. The title compound was prepared following the sequence of steps described in Example 58, except for substituting compound **98** for aminoethyl phosphonic acid diethyl ester. Purification of the crude final product on preparative thin layer chromatography eluted with 7% MeOH/CH₂Cl₂ provided 24 mg of the title compound **108**. ¹H NMR (300 MHz, CDCl₃) (5:1 diastereomeric ratio) δ 7.34 (t, 2H), 7.17 (m, 5H), 7.01 (t, 1H), 6.86 (d, 2H), 6.66 (t, 2H), 5.20 (bs, 4H), 4.96 (m, 1H), 4.63 (bs, 2H), 4.19 (m, 2H), 3.73 (s, 2H), 3.15 (m, 1H), 3.02 (m, 2H), 2.27 (m, 2H), 1.36 (d, 3H), 1.29 (d, 6H) 1.27 (m, 3H). ³¹P NMR (300 MHz, CDCl₃) δ 29.1, 27.4.

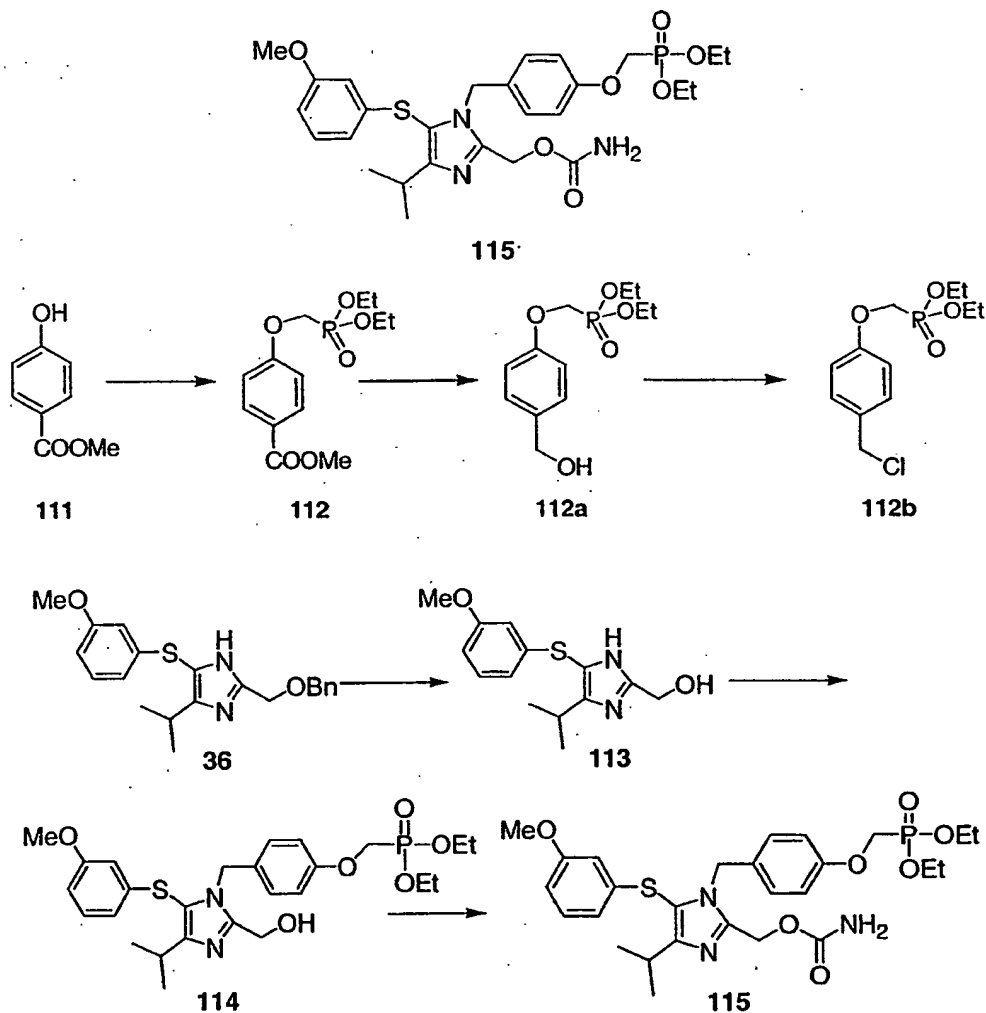
Example 60



Compound **109** was prepared from compound **29** following the sequence of steps described in Example 22. The title compound was prepared following the sequence of steps described in Example 58, except for substituting compound **109** for aminoethyl phosphonic

acid diethyl ester. Purification of the crude final product on silica gel eluted with 5-6% MeOH/CH₂Cl₂ provided 8 mg of the title compound. ¹H NMR (300 MHz, CDCl₃) (1.8:1 diastereomeric ratio) δ 7.31 (m, 2H), 7.16 (m, 5H), 7.01 (bs, 1H), 6.88 (d, 2H), 6.66 (bs, 2H), 5.21 (s, 2H), 5.20 (s, 2H), 4.69 (bd, 2H), 4.27 (bt, 1H), 4.12 (m, 3H), 3.75 (m, 2H), 3.16 (m, 1H), 2.99 (m, 2H), 2.11 (m, 2H), 1.30 (d, 6H), 1.22 (m, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 31.3, 30.8.

Example 61



Compound 112: A solution of methyl 4-hydroxybenzoate **111** (0.977 g, 6.42 mmol) and trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester (2.12 g, 7.06 mmol) in THF (50 mL) was treated with Cs₂CO₃ (4.18 g, 12.84 mmol). The resulting reaction mixture was stirred for 1 h at room temperature before it was partitioned between EtOAc and sat.

aqueous NH_4Cl and extracted with EtOAc (3x). The organic phase was washed with brine, dried over Na_2SO_4 , and evaporated under reduced pressure. Purification of the crude product on silica gel (eluted with 60-90% EtOAc/hexane) provided 1.94 g (quantitative) of methyl phosphonobenzoate compound **112** as a clear oil.

Alcohol 112a: A solution of **112** (1.94 g, 6.42 mmol) in Et_2O (40 mL) was treated with LiBH_4 (0.699 g, 32.1 mmol) and THF (10 mL). After the reaction mixture was stirred for 12 h at room temperature, the mixture was quenched with water and extracted with EtOAc (3x). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified on silica gel (eluted with 2-5% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give 1.48 g (84%) of alcohol compound **112a** as a colorless oil.

Chloride 112b: A solution of **112a** (315 mg, 1.15 mmol) in MeCN (6 mL) was treated with methanesulfonyl chloride (97.6 μL , 1.26 mmol), TEA (175 μL , 1.26 mmol), LiCl (74.5 mg, 1.72 mmol). After stirring at room temperature for 30 min., the mixture was concentrated under reduced pressure, partitioned between EtOAc and sat. NaHCO_3 , and extracted with EtOAc (3x). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. Purification of the crude product on silica gel (eluted with 2-4% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) provided 287 mg (85%) of chloride compound **112b** as a clear pale yellow oil.

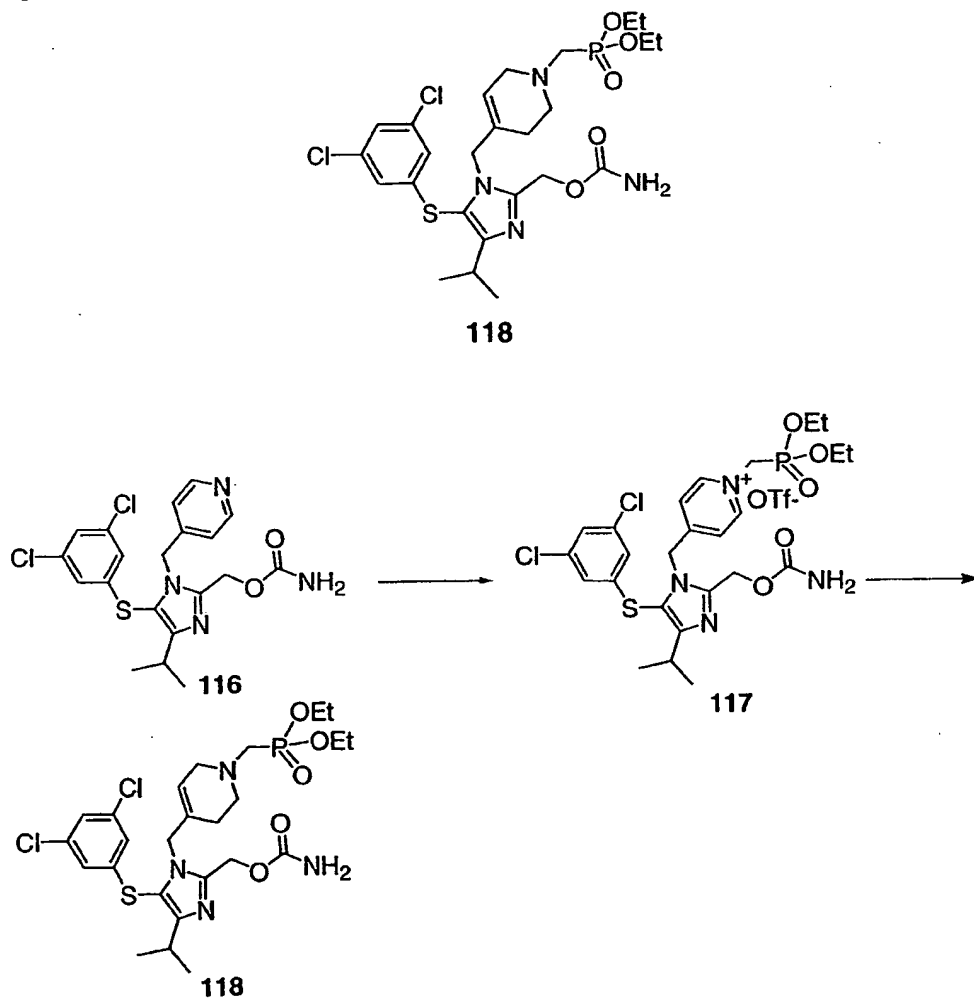
Alcohol compound 113: A solution of benzyl ether **36** (120 mg, 0.326 mmol) in EtOH (2 mL) was treated with conc. HCl (2 mL). After the reaction mixture was refluxed at 100°C for 1 day, the mixture was concentrated under reduced pressure, partitioned between EtOAc and sat. NaHCO_3 , and extracted with EtOAc (3x). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to provide the crude alcohol compound **113** (90 mg, 99%) as a white solid.

Compound 114: A solution of alcohol compound **113** (16.8 mg, 0.060 mmol) and chloride compound **112b** (21.1 mg, 0.072 mmol) in THF (1.5 mL) was treated with powder NaOH (3.5 mg, 0.090 mmol), lithium iodide (12.0 mg, 0.090 mmol), and tetrabutylammonium bromide (9.70 mg, 0.030 mmol). After the reaction mixture was stirred at room temperature for 15 h, the mixture was partitioned between EtOAc and sat. NH_4Cl . The organic phase was dried over Na_2SO_4 , filtered, and evaporated under reduced pressure. The crude product was purified on silica gel (eluted with 3-6% $\text{MeOH}/\text{CH}_2\text{Cl}_2$) to give compound **114** (19.7 mg, 61%) as a colorless oil.

Title compound 115: A solution of **114** (19.7 mg, 0.037 mmol) in CH_2Cl_2 (1 mL) was treated with trichloroacetyl isocyanate (13.2 μL , 0.111 mmol). After the reaction mixture was stirred at room temperature for 20 min, 2 mL of CH_2Cl_2 (saturated with NH_3) was added to

the mixture. After stirring at room temperature for 1 h, the mixture was bubbled with N₂ for 1 h. The mixture was then concentrated under reduced pressure and purified on silica gel (eluted with 4-6% MeOH/CH₂Cl₂) to give the titled compound **115** (18.5 mg, 87%) as a clear oil. ¹H NMR (300 MHz, CDCl₃) δ 7.09 (t, 1H), 6.90 (d, 2H), 6.78 (d, 2H), 6.63 (dd, 1H), 6.51 (dd, 1H), 6.40 (t, 1H), 5.15 (s, 2H), 5.11 (s, 2H), 4.70 (b, 2H), 4.21 (m, 6H), 3.70 (s, 3H), 3.22 (m, 1H), 1.36 (t, 6H), 1.29 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 19.2.

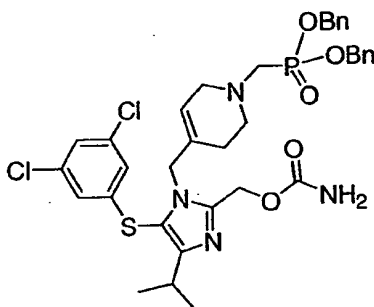
Example 62



A suspension of compound **116** (15mg, 0.03mmol) in acetone d-6 was treated with trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester (12mg, 0.04 mmol). The solution was stirred overnight at ambient temperature. Concentration afforded compound **117**. Compound **117** (22mg, 0.03mmol) was suspended in EtOH (2mL) and an excess of sodium borohydride (15mg, 0.39mmol) was added. The solution was stirred at room

temperature. After 30 minutes, sodium borohydride (15mg, 0.39mmol) was added again. Acetic acid (1ml) in EtOH was added 2 hours later followed by the addition of sodium borohydride (15mg, 0.39mmol). After 30 minutes, the solution was concentrated. The residue was dissolved in saturated aqueous NaHCO_3 and extracted with EtOAc (x3). The organic layers were washed with brine and dried over MgSO_4 . The solution was filtered, concentrated and purified using a TLC plate (5% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$) to give 14 mg (80%) of the desired product. ^1H NMR (CDCl_3 , 500MHz): 7.13 (s, 1H), 6.83 (s, 2H), 5.16 (s, 2H), 5.01 (s, 1H), 4.51 (s, 2H), 4.14 (m, 4H), 3.15 (m, 1H), 3.00 (s, 2H), 2.80 (d, 2H), 2.68 (t, 2H), 1.97 (s, 2H), 1.33 (t, 6H), 1.29 (d, 6H).

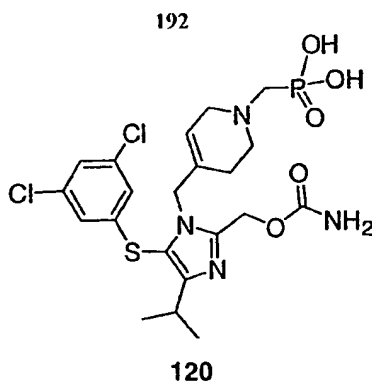
Example 63



119

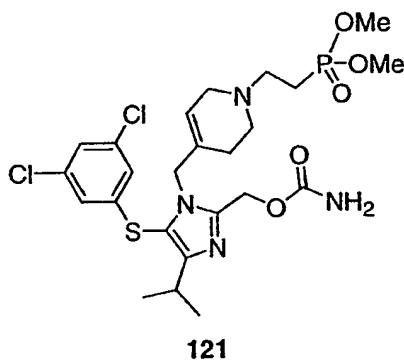
Title compound **119** was prepared following the sequence of steps described in Example 62 by substituting trifluoro-methanesulfonic acid bis-benzyloxy-phosphorylmethyl ester for trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester. Purification of the crude final product on silica gel eluted with (2.5% - 5% $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2$) provided 71 mg (65%) of the title compound. ^1H NMR (CDCl_3 , 500 MHz): 7.35 (s, 10H), 7.11 (s, 1H), 6.82 (s, 2H), 5.16 (s, 2H), 5.04 (d, 4H), 4.99 (s, 1H), 4.49 (s, 2H), 3.15 (m, 1H), 2.96 (s, 2H), 2.81 (d, 2H), 2.63 (t, 2H), 1.91 (s, 2H), 1.29ppm(d, 6H).

Example 64

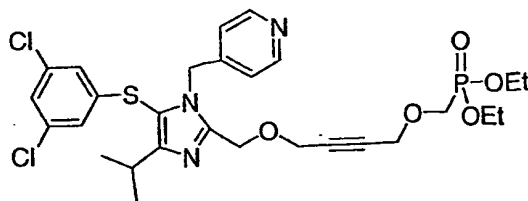
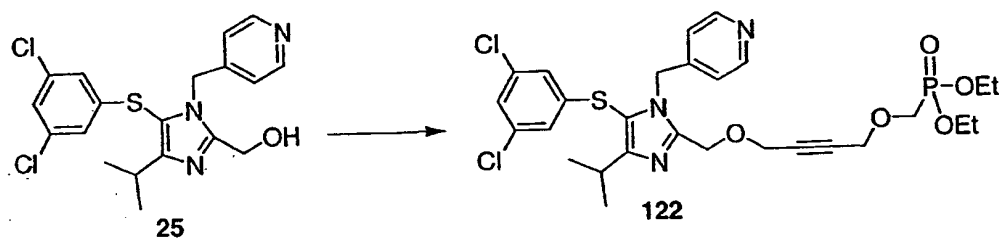


Compound **119** was stirred in 4M HCl/dioxane overnight at ambient temperature. The mixture was concentrated and purified using HPLC (20% CH₃CN/H₂O) to provide 20 mg of the title compound **120**. ¹H NMR (CD₃OD₃, 500 MHz) 7.33 (s, 1H) 7.00 (s, 2H), 5.22 (s, 2H), 5.12 (s, 1H), 4.79 (s, 2H), 3.80 (s, 2H), 3.49 (s, 2H), 3.23 (m, 2H), 3.21 (m, 1H), 2.40 (s, 2H), 1.28 (d, 6H).

Example 65



Compound **121** was prepared following the sequence of steps described in Example 62 by substituting trifluoro-methanesulfonic acid dimethoxy-phosphorylethyl ester for trifluoro-methanesulfonic acid diethoxy-phosphorylmethyl ester. Purification of the crude final product on TLC plate eluted with (5% CH₃OH/CH₂Cl₂) provided 11 mg (65%) of the title compound. ¹H NMR (CDCl₃, 500 MHz): 7.34 (d, 2H), 7.20 (d, 2H), 7.19 (d, 1H), 7.13 (s, 1H), 6.83 (s, 2H), 5.18 (s, 2H), 5.03 (s, 1H), 4.98 (m, 1H), 4.52 (s, 2H), 4.22 (m, 2H), 3.15 (m, 1H), 2.91 (s, 2H), 2.81 (s, 2H), 2.54 (s, 2H), 2.29 (m, 2H), 2.01 (d, 2H), 1.56 (d, 3H), 1.38 (d, 3H), 1.28 (q, 3H), 1.28 (d, 6H).

Example 66**122****25****122**

A solution of **25** (33.2 mg, 0.081 mmol) in DMF (3 mL) under N₂ at 0°C was treated with NaH. After stirring at 0°C for 10 min, **95** (23 mg, 0.077 mmol) was added, and the resulting mixture was slowly raised to room temperature and stirred at room temperature for 8 h. The mixture was then poured into water, and extracted with EtOAc. The combined organic layers were washed with brine, dried (Na₂SO₄), filtered, and evaporated under reduced pressure. The crude product was purified on TLC plate (eluted with 3% MeOH/CH₂Cl₂) to provide 17.9 mg of the title compound **122**. ¹H NMR (500 MHz, CDCl₃) δ 8.45 (d, 2H), 7.04 (t, 1H), 6.88 (d, 2H), 6.67 (d, 2H), 5.24 (s, 2H), 4.67 (s, 2H), 5.02 (m, 1H), 4.27 (bs, 2H), 4.22 (bs, 2H), 4.19 (m, 4H), 3.82 (m, 2H), 3.16 (m, 1H), 1.35 (t, 6H), 1.30 (d, 6H). ³¹P NMR (300 MHz, CDCl₃) δ 20.8.

Example 67: Anti-HIV-1 Cell Culture Assay

The assay is based on quantification of the HIV-1-associated cytopathic effect by a colorimetric detection of the viability of virus-infected cells in the presence or absence of tested inhibitors. The HIV-1-induced cell death is determined using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) which is converted only by intact cells into a product with specific absorption characteristics as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR (1989) *J Natl Cancer Inst* 81, 577.

Assay protocol for determination of EC₅₀:

1. Maintain MT2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Infect the cells with the wild-type HIV-1 strain IIIB (Advanced Biotechnologies, Columbia, MD) for 3 hours at 37°C using the virus inoculum corresponding to a multiplicity of infection equal to 0.01.
3. Distribute the infected cells into a 96-well plate (20,000 cells in 100 µL/well) and add various concentrations of the tested inhibitor in triplicate (100 µL/well in culture media). Include untreated infected and untreated mock-infected control cells.
4. Incubate the cells for 5 days at 37°C.
5. Prepare XTT solution (6 ml per assay plate) at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in water-bath for 5 min at 55°C. Add 50 µL of N-methylphenazonium methasulfate (5 µg/mL) per 6 mL of XTT solution.
6. Remove 100 µL media from each well on the assay plate.
7. Add 100 µL of the XTT substrate solution per well and incubate at 37°C for 45 to 60 min in a CO₂ incubator.
8. Add 20 µL of 2% Triton X-100 per well to inactivate the virus.
9. Read the absorbance at 450 nm with subtracting off the background absorbance at 650 nm.
10. Plot the percentage absorbance relative to untreated control and estimate the EC₅₀ value as drug concentration resulting in a 50% protection of the infected cells.

Example 68: Cytotoxicity Cell Culture Assay (Determination of CC₅₀):

The assay is based on the evaluation of cytotoxic effect of tested compounds using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfohenyl)-2H-tetrazolium-5-carboxanilide (XTT) as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR (1989) *J Natl Cancer Ins* 81, 577.

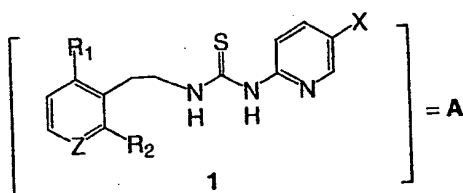
Assay protocol for determination of CC₅₀:

1. Maintain MT-2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Distribute the cells into a 96-well plate (20,000 cell in 100 µL media per well) and add various concentrations of the tested compound in triplicate (100 µL/well). Include untreated control.

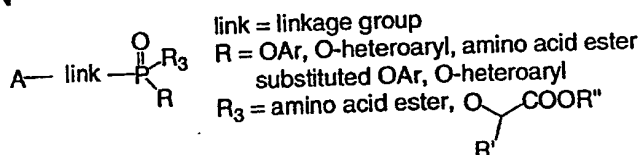
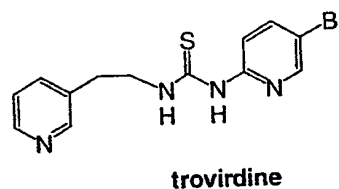
3. Incubate the cells for 5 days at 37°C.
4. Prepare XTT solution (6 ml per assay plate) in dark at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in a water-bath at 55°C for 5 min. Add 50 µL of N-methylphenazonium methasulfate (5 µg/mL) per 6 mL of XTT solution.
5. Remove 100 µL media from each well on the assay plate and add 100 µL of the XTT substrate solution per well. Incubate at 37°C for 45 to 60 min in a CO₂ incubator.
6. Add 20 µL of 2% Triton X-100 per well to stop the metabolic conversion of XTT.
7. Read the absorbance at 450 nm with subtracting off the background at 650 nm.
8. Plot the percentage absorbance relative to untreated control and estimate the CC50 value as drug concentration resulting in a 50% inhibition of the cell growth. Consider the absorbance being directly proportional to the cell growth.

PETT-like phosphonate NNRTI compounds

The PETT class of compound has demonstrated activity in inhibiting HIV replication. The present invention provides novel analogs of PETT class of compound. Such novel PETT analogs possess all the utilities of PETT and optionally provide cellular accumulation as set forth below.



R₁ = H, F, Cl, OMe Z = CH
 R₂ = H, F, Cl, OMe Z = N when R₁ and R₂ are H
 X = Cl, Br, CN



The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.

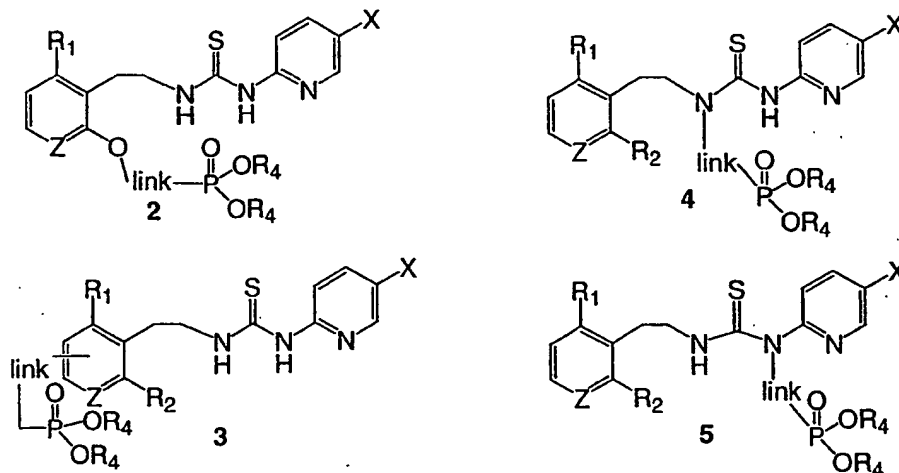
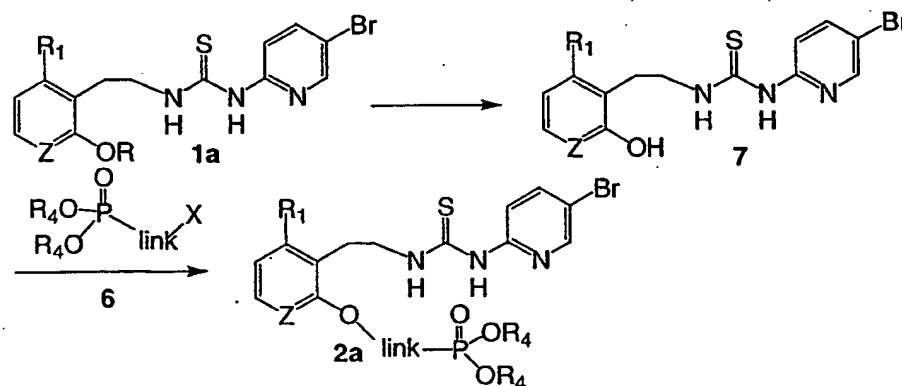


Figure 2

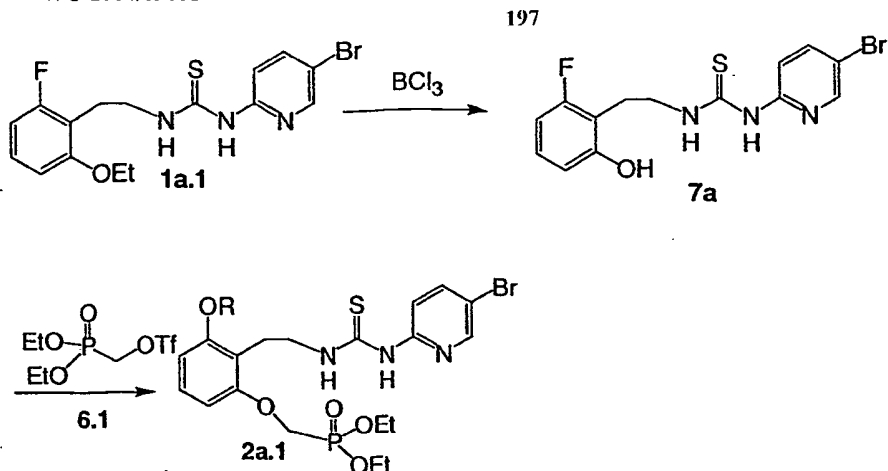
PETT 1 compounds, analogs of trovirdine, are obtained following the procedures described in WO/9303022 and *J. Med. Chem.* **1995**, 38, 4929-4936 and **1996**, 39, 4261-4274. Preparation of PETT-like phosphonate NNRTI compounds, e.g. phosphonate analog type 2 is outlined in Scheme 1. PETT analog 1a is obtained following the above mentioned literature procedure. Alkyl group of 1a is then removed using such as, for example BCl_3 to give phenol 7, many examples are described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3rd Edition, John Wiley and Sons Inc. Conversion of 7 to the desired phosphonate analogs is realized by treatment of 7 with the phosphonate reagent 6 under suitable conditions.

For example (Example 1), PETT 1a is treated with BCl_3 to give phenol 7. Treatment of 7 with phosphonate 6.1 in the presence of base, for example, Cs_2CO_3 , affords the phosphonate 2a.1. Using the above procedure but employing a different phosphonate reagent 5 in place of 6.1, corresponding products 2 with different linking groups are obtained.

Scheme 1



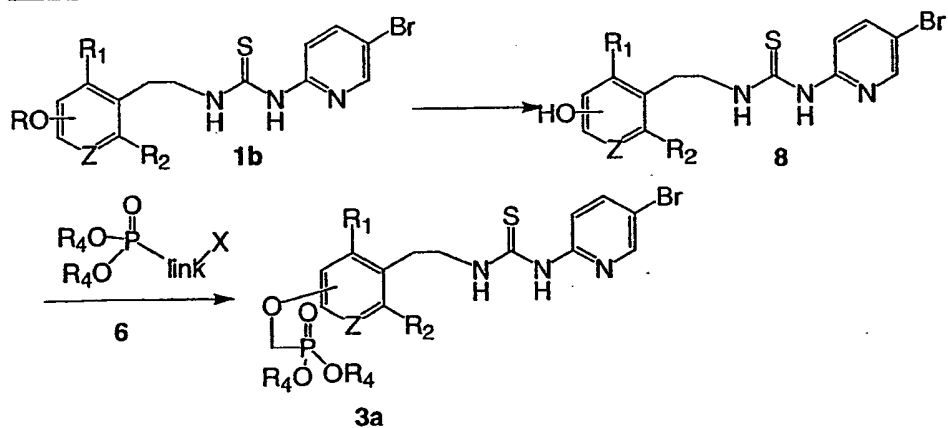
Example 1



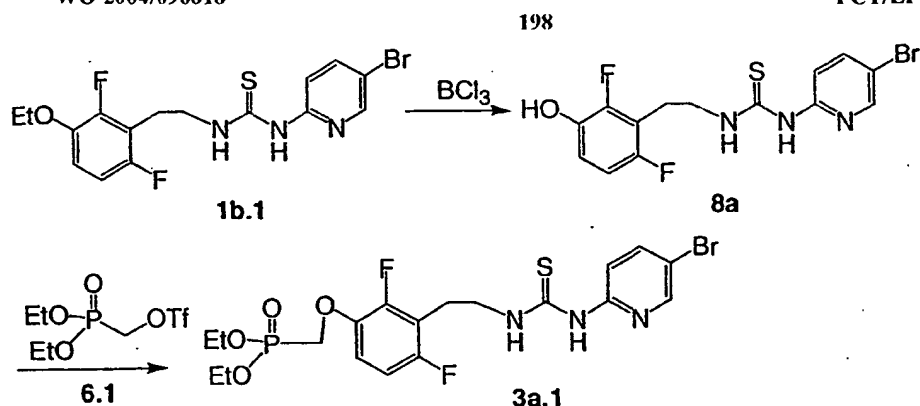
Scheme 2 shows the preparation of phosphonate type 3 in Figure 2. PETT 1b is obtained as described in WO/9303022 and *J. Med. Chem.* 1995, 38, 4929-4936 and 1996, 39, 4261-4274. Alkyl group of 1b is then removed using such as, for example BCl_3 to give phenol 8, many examples are described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3rd Edition, John Wiley and Sons Inc. Conversion of 8 to the desired phosphonate analogs is realized by treatment of 8 with the phosphonate reagent 6 under suitable conditions.

For example (Example 1), PETT 1a is treated with BCl_3 to give phenol 7. Treatment of 7 with triflate methyl phosphonic acid diethyl ester 6.1 in the presence of base, for example, Cs_2CO_3 , affords the phosphonate 2a.1. Using the above procedure but employing a different phosphonate reagent 6 in place of 6.1, corresponding products 3 with different linking groups are obtained.

Scheme 2

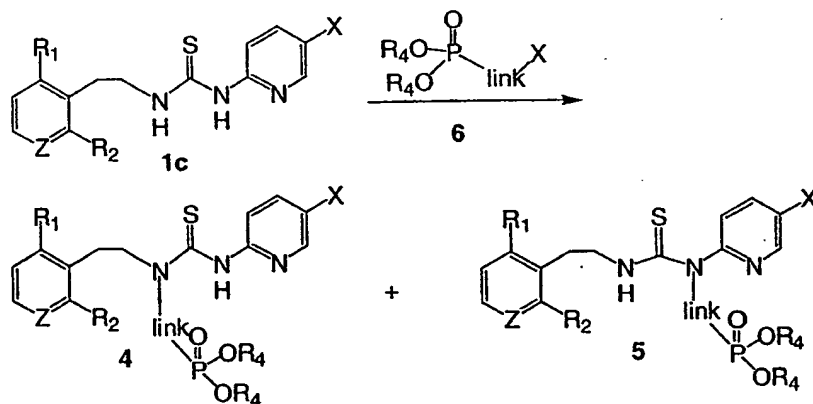


Example 2

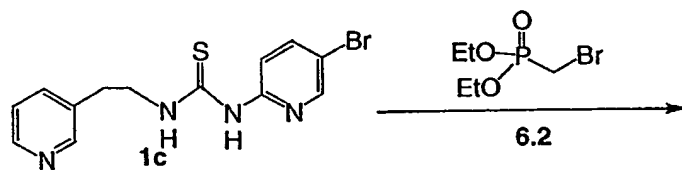


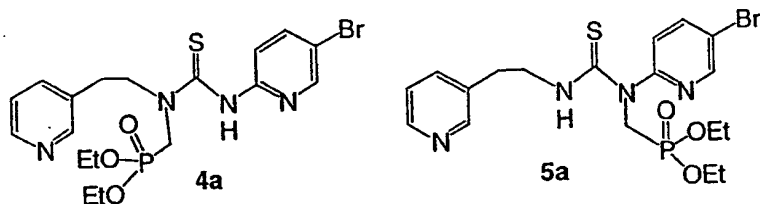
Scheme 3 shows of the preparation of the phosphonate linkage of type 4 and 5 to PETT. PETT 1c is first treated with a suitable base to remove the thiourea proton, the product is then treated with 1 equivalent of a phosphonate reagent 5 bearing a leaving group such as, for example, bromine, mesyl, tosyl etc to give the alkylated product 4 and 5. The phosphonates 4 and 5 are separated by chromatography. For example (Example 3), PETT 1, in DMF, is treated with sodium hydride followed by one equivalent of bromomethyl phosphonic acid dibenzyl ester 6.2 to give phosphonate 4a and 5a. Phosphonate product 4a and 5a are then separated by chromatography to give pure 4a and 5a respectively. Using the above procedure but employing a different phosphonate reagent 5 in place of 6.2, corresponding products 4 and 5 with different linking groups are obtained.

Scheme 3



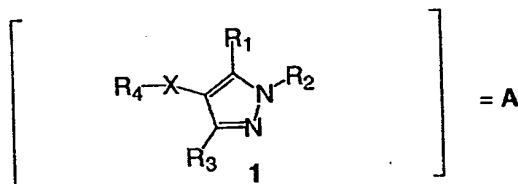
Example 3





Pyrazole-like phosphonate NNRTI compounds

The present invention includes pyrazole-like phosphonate NNRTI compounds and describes methods for their preparation. Pyrazole-like phosphonate NNRTI compounds are potential anti-HIV agents.



R_1 , R_2 , R_3 and R_4 , X are defined as described in Patent WO02/04424.

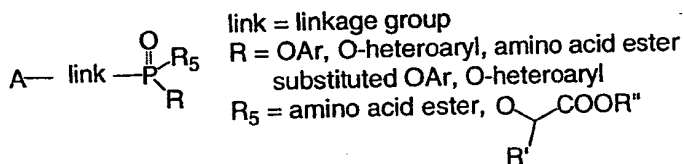
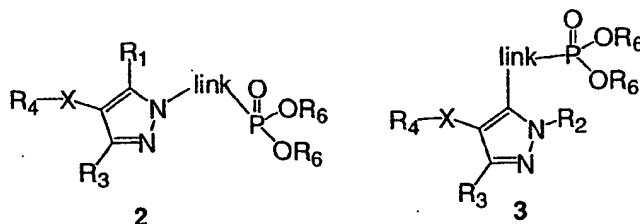


Figure 1

A link group includes a portion of the structure that links two substructures, one of which is pyrazole class of HIV inhibiting agents having the general formula shown above, the other is a phosphonate group bearing the appropriate R and R_5 groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

Pyrazole class of compounds has shown to be inhibitors of HIV RT. The present invention provides novel analogs of pyrazole class of compound. Such novel pyrazole analogs possess all the utilities of pyrazoles and optionally provide cellular accumulation as set forth below.

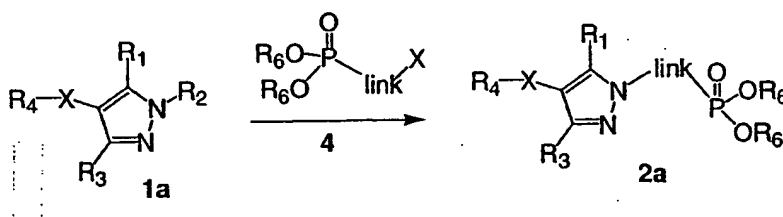
The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2, where R_1 , R_2 , R_3 , R_4 and X are as described in WO02/04424.

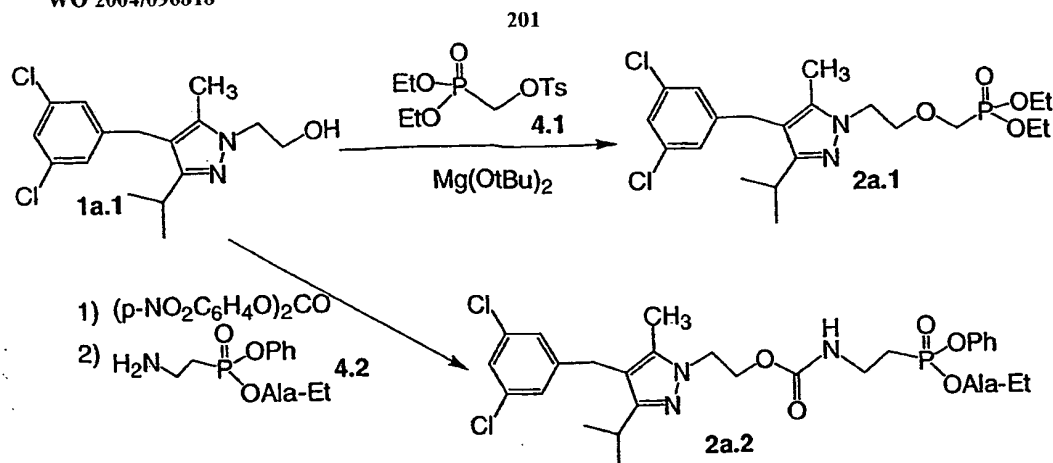
Figure 2

Pyrazole **1** is obtained following the procedures described in WO02/04424.

Preparation of phosphonate analog type **2** is outlined in Scheme 1. Pyrazole analog **1a**, which R_2 bears a function group can be used as attaching site for phosphonate prodrug, is obtained as described in the above mentioned literature. Conversion of **1a** to the desired phosphonate analogs is realized by treatment of **2a** with the phosphonate reagent **4** under suitable conditions.

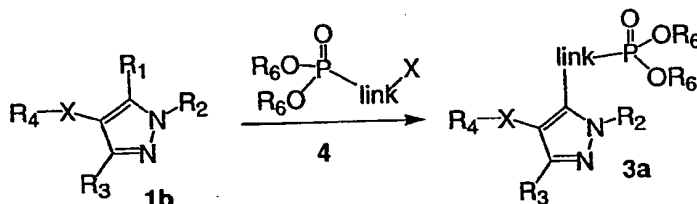
For example (Example 1), treatment of pyrazole **1a.1** with phosphonate **4.1** in the presence of base, for example, $Mg(OtBu)_2$, affords the phosphonate **2a.1**. Using the above procedure but employing a different phosphonate reagent **4** in place of **4.1**, corresponding products **2a** with different linking groups are obtained. Alternatively, activation of the hydroxyl group with bis(4-nitrophenyl) carbonate, following by treatment with amino ethyl phosphonate **4.2** provides phosphonate **2a.2**. Using different phosphonate **4** in place of **4.2** and/or different methods for linking them together affords **2** with different linker.

Scheme 1Example 1

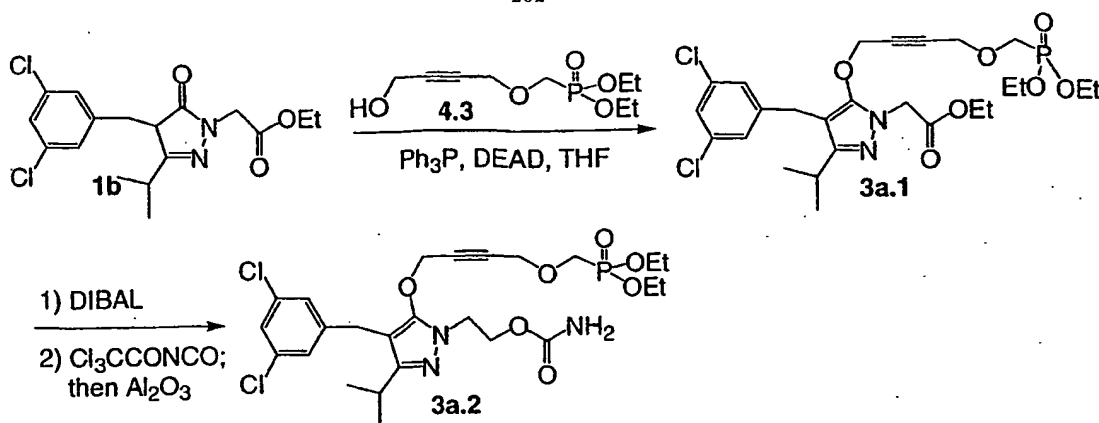


Scheme 2 shows the preparation of phosphonate type 3 conjugate to pyrazole in Figure 2. Pyrazole **1b**, bearing a functional group at position R_1 can be used as attaching site for phosphonate prodrug, is obtained as described in WO02/04424. Conversion of **1b** to the desired phosphonate **3** analogs is realized by treatment of **1b** with the phosphonate reagent **4** under suitable conditions. For example (Example 2), pyrazole **1b** reacts with phosphonate **4.3** in the presence of triphenyl phosphine and DEAD in THF, affords the phosphonate **3a.1**. Phosphonate **3a.2** is obtained by first reducing the ester to alcohol, and then by treating the resulting alcohol with trichloroacetyl isocyanate, and followed by alumina. Using the above procedure but employing a different phosphonate reagent **4** in place of **4.3**, corresponding products **3** with different linking groups are obtained.

Scheme 2

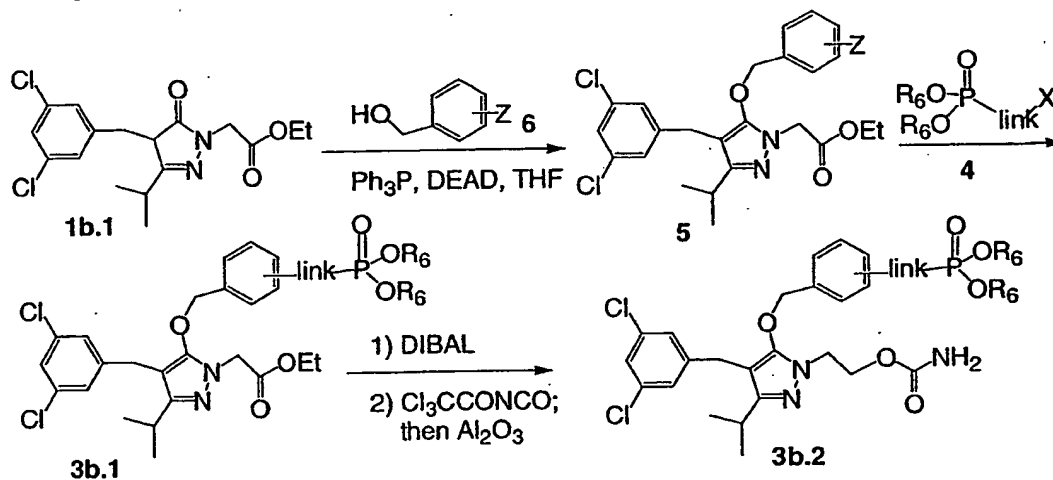


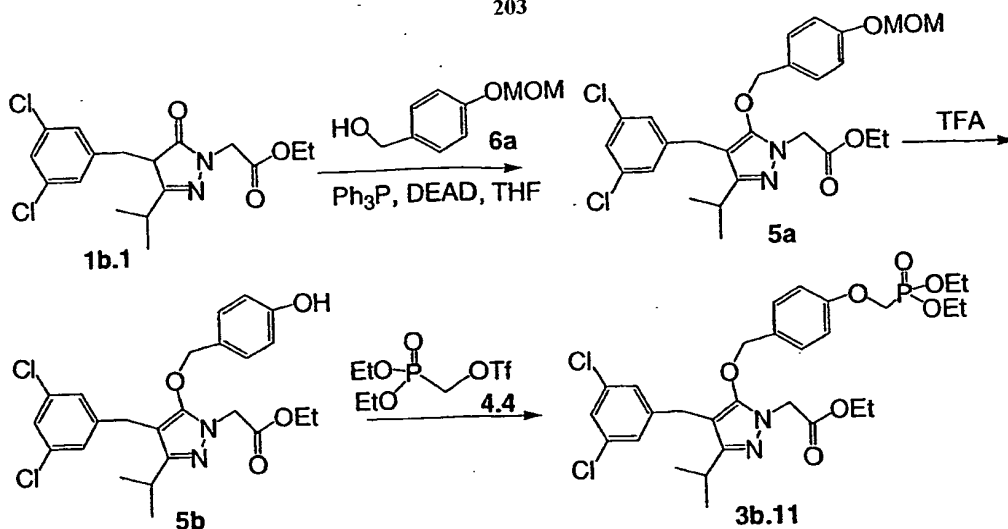
Example 2



Alternatively, as shown in Example 3, reaction of pyrazolone **1b.1** with a moiety bearing a protected function group which can be used to attach phosphonate, for example benzyl alcohol with a protected hydroxyl or amino group, under Mitsunobu condition affords compound **5**. The protecting group of **Z** is then removed, and the resulting product is reacted with phosphonate reagent yields phosphonate **3b.1**. Phosphonate **3b.1** is converted to phosphonate **3b.2** following the procedures described Example 2. Reaction of pyrazolone **1b.1** with benzyl alcohol **6b** with Ph₃P/DEAD produces **5a**. The protecting group MOM- is then removed with TFA to give phenol **5b**. Treatment of phenol with triflate methyl phosphonic acid dibenzyl ester **4a** to give phosphonate **3b.11**, which is also converted to **3b.2** type of compound.

Example 3





Urea-PETT-like phosphonate NNRTI compounds

The present invention includes and describes Urea-PETT-like phosphonate NNRTI compounds and methods for their preparation. Urea-PETT-like phosphonate NNRTI compounds are potential anti-HIV agents.

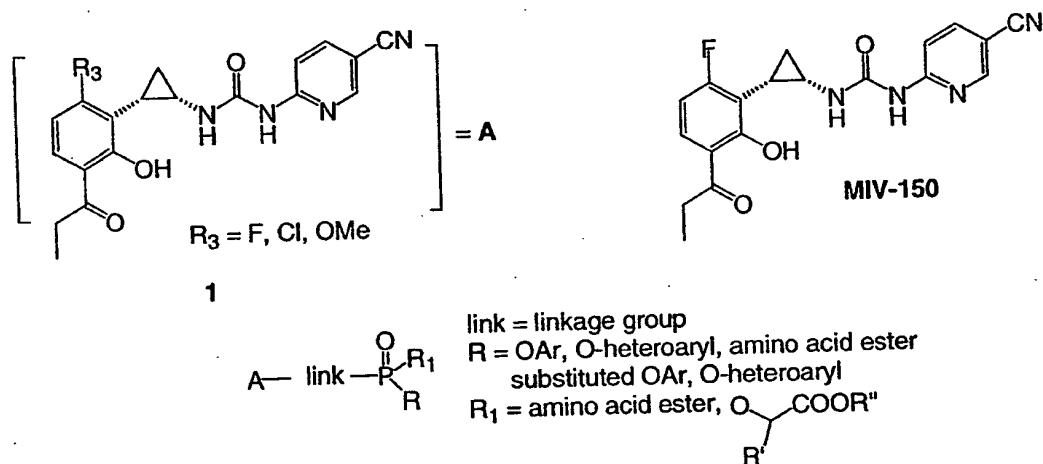


Figure 1

A link group includes a portion of the structure that links two substructures, one of which is urea-PETT class of HIV inhibiting agents having the general formula shown above, the other is a phosphonate group bearing the appropriate R and R₁ groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

Urea-PETT class of compound has demonstrated activity in inhibiting HIV replication. The present invention provides novel analogs of urea-PETT class of compound. Such novel

urea-PETT analogs possess all the utilities of urea-PETT and optionally provide cellular accumulation as set forth below.

The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.

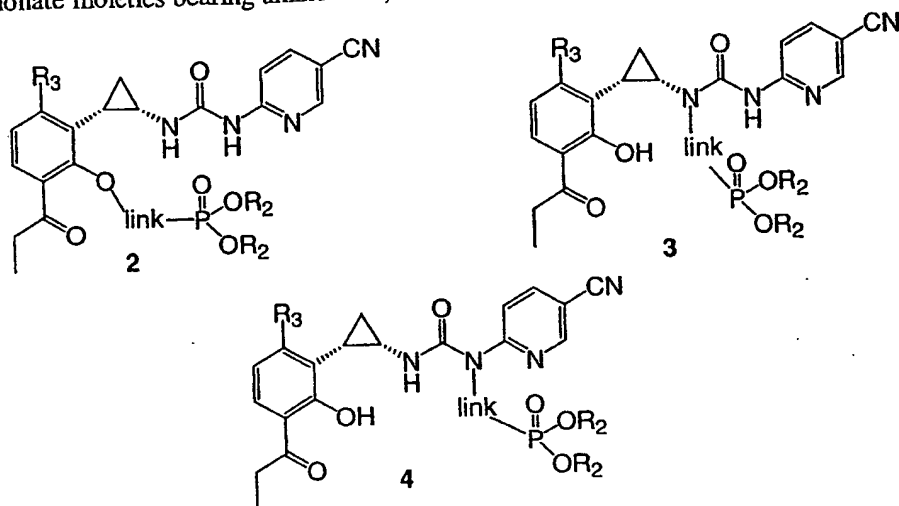
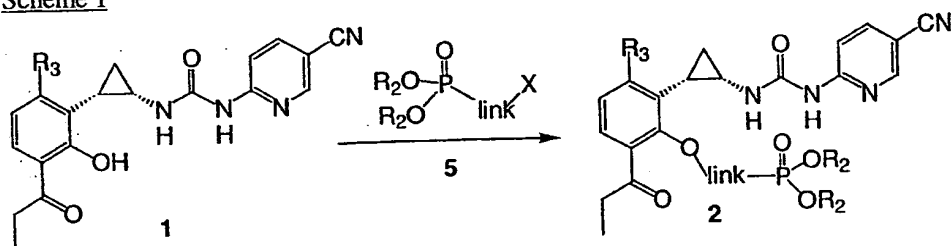


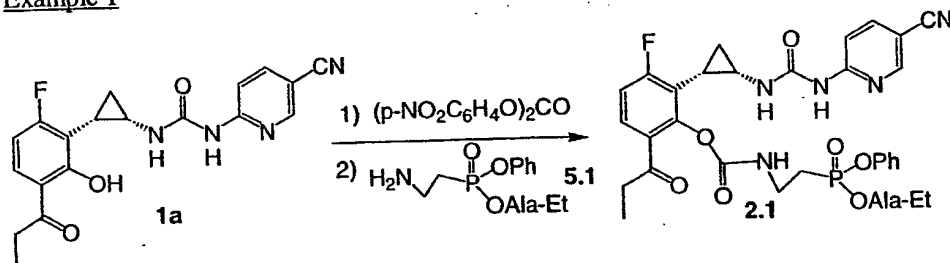
Figure 2

Preparation of phosphonate analog type 2 is outlined in Scheme 1. Urea-PETT 1 is described in US Patent No. 6486183 and *J. Med. Chem.* 1999, 42, 4150-4160. Conversion of 1 to the desired phosphonate analogs is realized by treatment of 1 with the phosphonate reagent 5 under suitable conditions. For example (Example 1), urea-PETT 1a is activated as it *p*-nitro-phenol carbonate by reacting with bis(4-nitrophenyl)carbonate. Reaction of the resulting carbonate with amino ethyl phosphonate 5.1 in the presence of base, for example, Hunig's base, affords the phosphonate 2.1.

Scheme 1



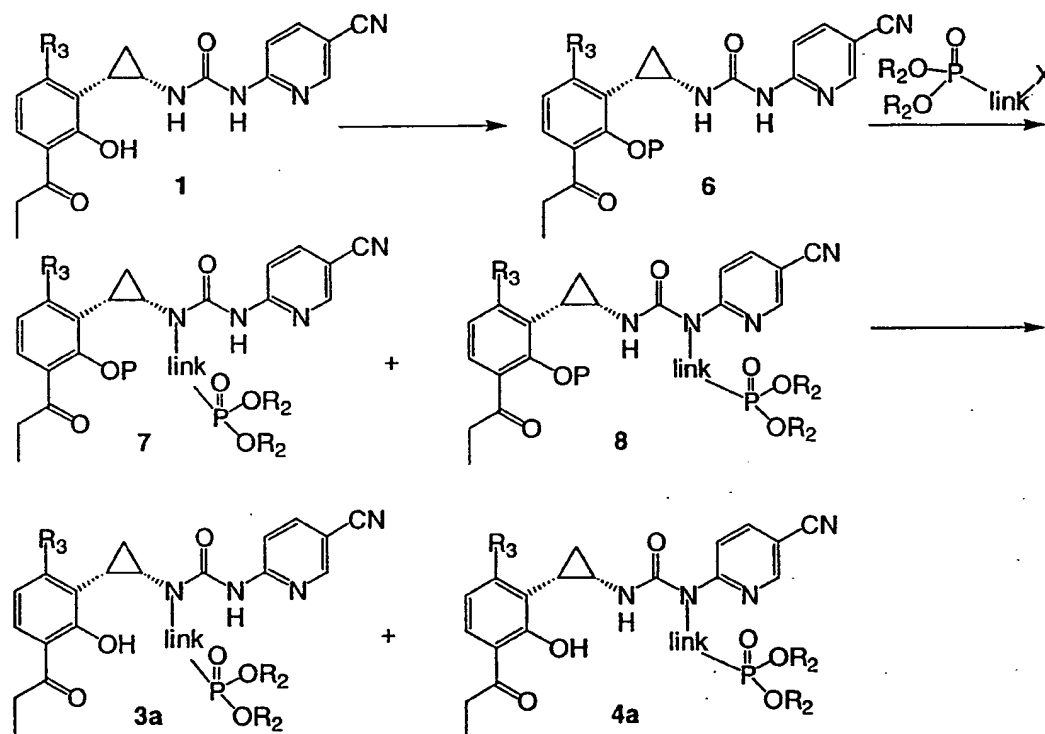
Example 1

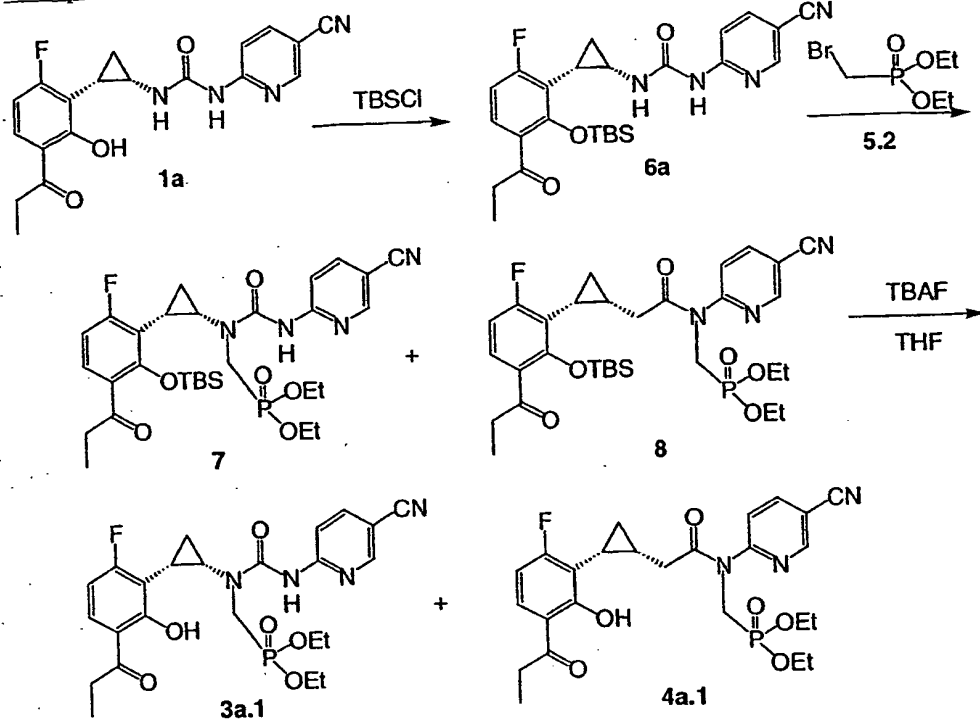


Scheme 2 shows of the preparation of the phosphonate linkage of type 2 and 3 to urea-PETT. The hydroxyl group of urea-PETT 1 is protected with a suitable protecting group, for example, trityl, silyl, benzyl or MOM- etc to give 6 as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3rd Edition, John Wiley and Sons Inc. The resulting protected urea-PETT 6 is first treated with a suitable base to remove the urea proton, the product is then treated with 1 equivalent of a phosphonate reagent 5 bearing a leaving group such as, for example, bromine, mesyl, tosyl etc to give the alkylated product 7 and 8. The phosphonates 7 and 8 are separated by chromatography and independently deprotected using conventional conditions described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3rd Edition, John Wiley and Sons Inc. p116-121. For example (Example 2), urea-

PETT 1 is protected as t-butyl dimethyl silyl ether 6a by reacting with TBSCl and imidazole. Compound 6a, in DMF, is treated with sodium hydride followed by one equivalent of bromomethyl phosphonic acid dibenzyl ester 5.2 to give phosphonate 7a and 8a respectively. phosphonates 7a and 8a are separated by chromatography, and then independently deprotected by treatment with TBAF in an aprotic solvent such as THF or acetonitrile to give 3a and 4a respectively in which the linkage is a methylene group. Using the above procedure but employing a different phosphonate reagent 5 in place of 5.2, corresponding products 3 and 4 with different linking groups are obtained.

Scheme 2



Example 2Nevaripine-like phosphonate NNRTI compounds

The present invention describes methods for the preparation of phosphonate analogs of nevaripine class of HIV inhibiting agents shown in Figure 1 that are potential anti-HIV agents.

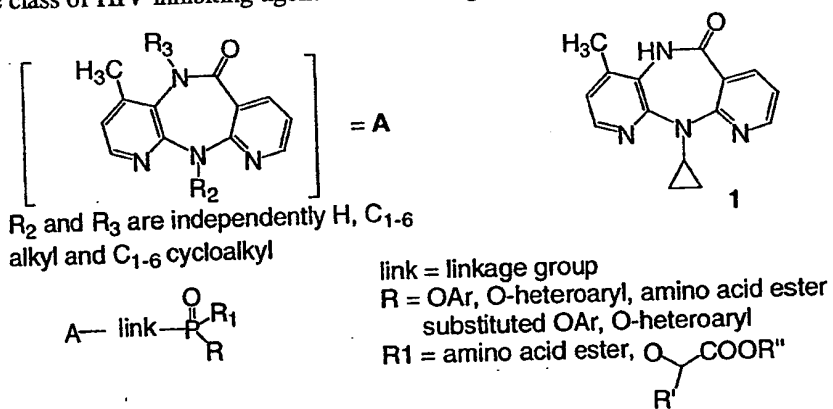
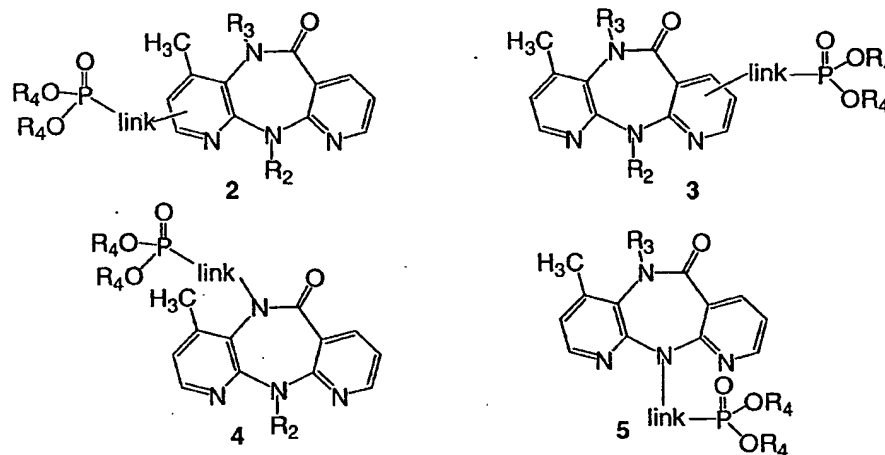


Figure 1

A link group includes a portion of the structure that links two substructures, one of which is nevirapine class of HIV inhibiting agents having the general formula shown above, the other is a phosphonate group bearing the appropriate R and R1 groups. The link has at least one uninterrupted chain of atoms other than hydrogen. Nevirapine-type compounds are inhibitors of HIV RT, and nevirapine is currently used in clinical for treatment of HIV infection and AIDs. The present invention provides novel analogs of nevirapine class of compound. Such novel nevirapine analogs possess all the utilities of nevirapine and optionally provide cellular accumulation as set forth below.

The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.

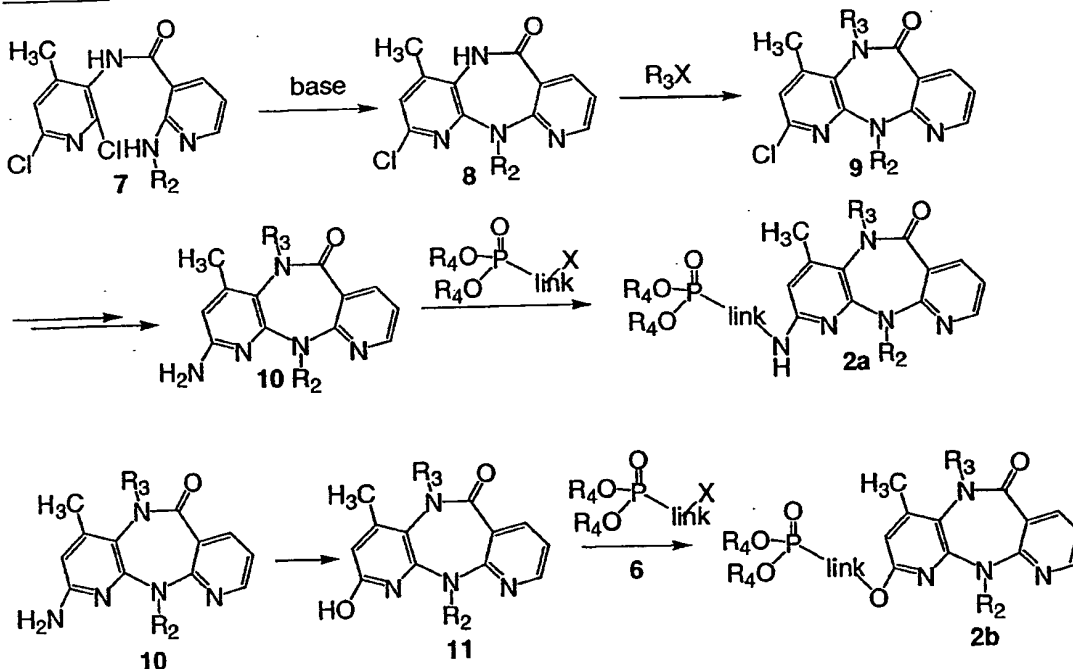
**Figure 2**

Compound 1 is synthesized as described in US Patent No. 5366972 and *J. Med. Chem.* **1991**, *34*, 2231. Preparation of phosphonate analog 2 is outlined in Scheme 1 and 2. Amide 7 is prepared as described in US Patent No. 5366972 and *J. Med. Chem.* **1998**, *41*, 2960-2971 and 2972-2984. Amide 7 is converted to dipyrindodizaepinone 10 following the procedures described in US Patent No. 5366972 and *J. Med. Chem.* **1998**, *41*, 2960-2971 and 2972-2984. Namely, treatment of dipyridine amide 7 with base provides the dipyrindodizaepinone 8. Alkylation of the amide N- is achieved with base and alkyls bearing a leaving group, such as, for example, bromide, iodide, mesylate etc. Displacement of chloride with *p*-methoxybenzylamine, followed by removal of the *p*-methoxybenzyl group affords amine 10.

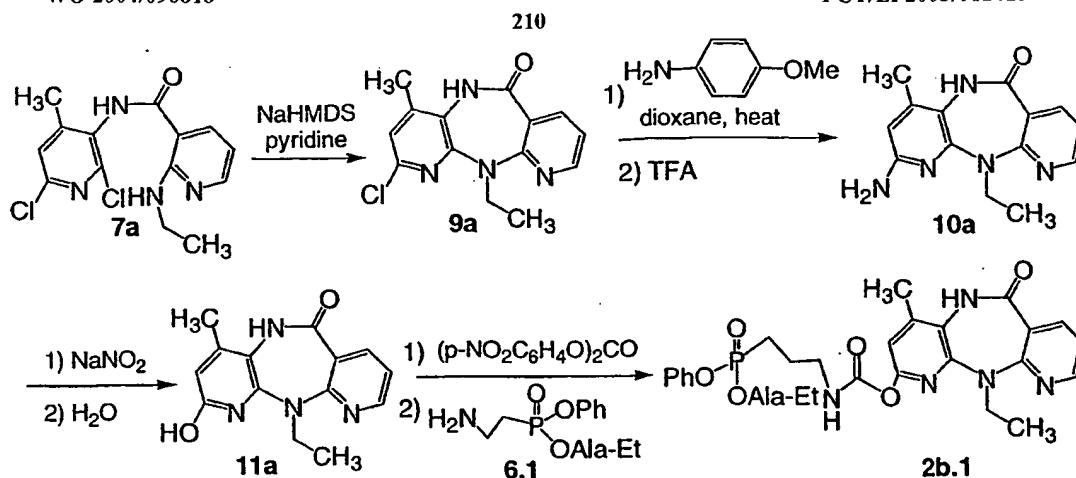
The amine group serves as the attachment site for introduction of a phosphonate group. Reaction of amine **10** with reagent **6** provides **2** with different linker attached to amine.

Alternatively (Scheme 2), amine **10** is transformed to phenol **11** as described in *J. Med. Chem.* **1998**, *41*, 2972-2984, many examples are also described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2nd Ed. the hydroxyl group then serves as the linking site for a suitable phosphonate group. Reaction of amine **11** with reagent **6** provides **2** with different linker attached to hydroxyl group. For example (Example 1), amide **7a**, obtained as described in *J. Med. Chem.* **1998**, *41*, 2960-2971 and 2972-2984, is treated with sodium hexamethyldisilazane in pyridine to give diazepinone **9a**. Amine **10a** is synthesized from **9a** by displacement of the chloride with *p*-methoxybenzylamine followed by removal of the protecting group of amine. Diazotization of the amine **10a** and subsequent in situ conversion to hydroxy yields phenol **11a**. Phosphonate with different linker is then able to be attached at the phenol site. For example, the phenol is activated as *p*-nitro-benzyl carbonate, subsequent treatment with amino ethyl phosphonate **6.1** in the presence of Hunig's base affords carbamate **2b.1**.

Scheme 1



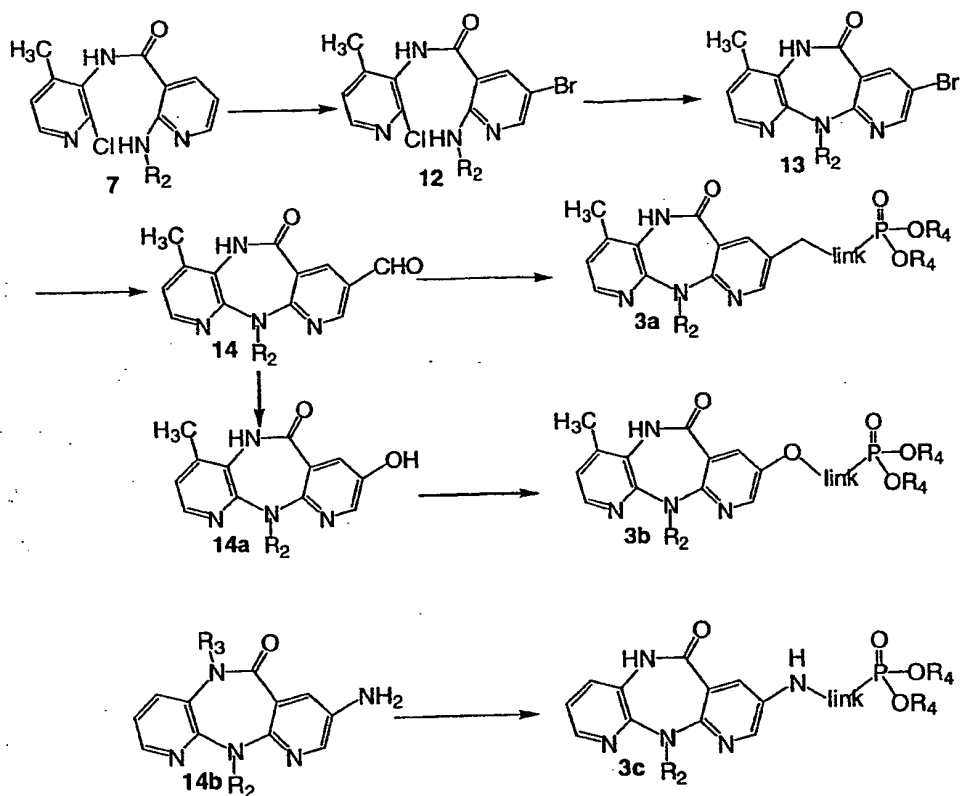
Example 1



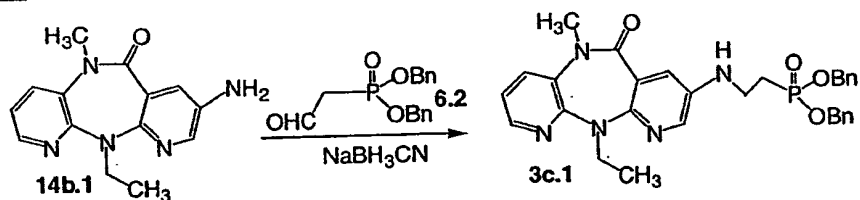
Scheme 2 shows the preparation of phosphonate conjugates compounds type 3 in Figure 2. Diazapinone 13 is obtained from dipyrido amide 7 following the procedure described in *J. Med. Chem.* **1998**, *41*, 2960-2971 and 2972-2984, which is then converted to aldehyde 14 and phenol 14a following the procedures in the same literature. Aldehyde 14 and phenol 14a are then converted to 3a and 3b respectively by reacting with suitable phosphonate reagents 6. Amine 14b is obtained using the method described in *J. Med. Chem.* **1998**, *41*, 2960-2971, which is converted to phosphonate 3c.

For example (Example 2), amine 14b.1, obtained by using the procedures described in *J. Med. Chem.* **1998**, *41*, 2960-2971, reacts with phosphonic acid dibenzyl ester 6.2 under reductive amination conditions to give phosphonate 3c.1.

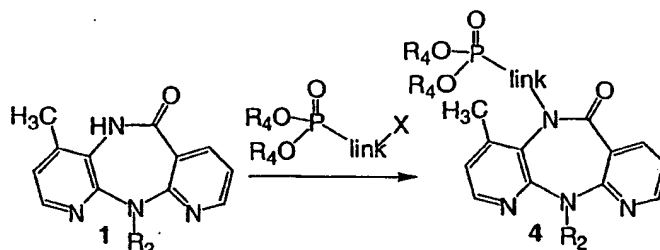
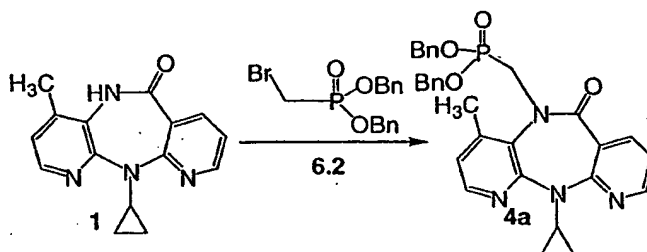
Scheme 2



Example 2

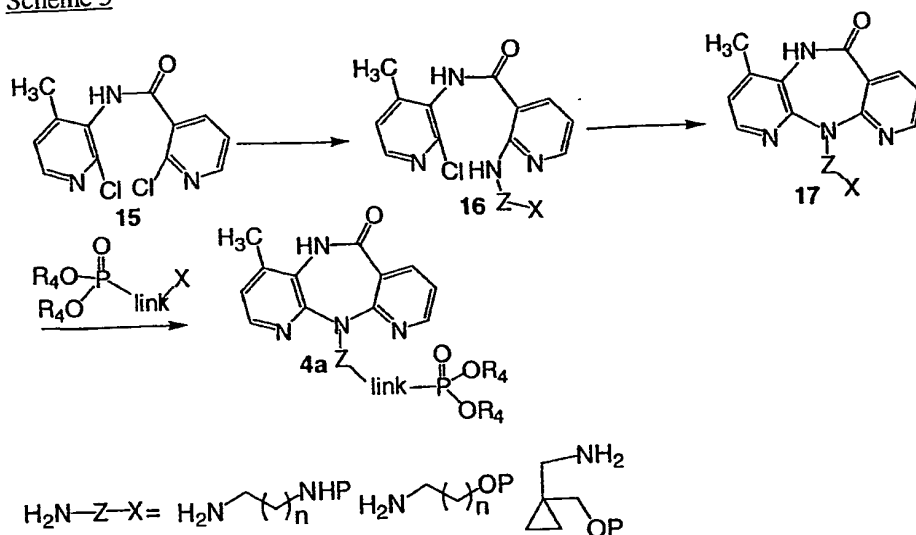


Preparation of phosphonate analog type 4 in Figure 2 is shown in Scheme 3. nevirapine analog **1** is dissolved in suitable solvent such as, for example, DMF or other protic solvent, and treated with the phosphonate reagent **9**, bearing a leaving group, such as, for example, bromine, mesyl, tosyl, or triflate, in the presence of a suitable organic or inorganic base, to give phosphonate **4**. For example, **1** was dissolved in DMF, is treated with sodium hydride and 1 equivalent of bromomethyl phosphonic acid dibenzyl ester **6.2** to give phosphonate **4a** in which the linkage is a methylene group.

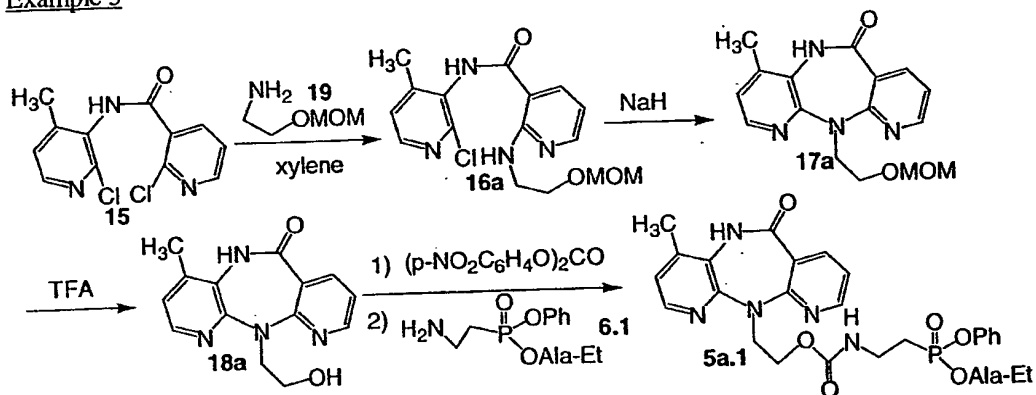
Scheme 3Example 3

Scheme 4 shows the preparation of phosphonate type 5 in Figure 2. Amine 15 is prepared according to the procedures described in US Patent No. 5366972 and *J. Med. Chem.* 1998, 41, 2960-2971 and 2972-2984. Substituted alkyl amines, which bearing a protected amino or hydroxyl group, or a precursor of amino group, are used in displacement of alkyls described in US Patent No. 5366972 and *J. Med. Chem.* 1998, 41, 2960-2971 and 2972-2984, react with the chloropyridine 15 in the presence of base to give amine 16. These alkyl amines include but not limit to examples in Scheme 4. These substituted alkyl amines are obtained from commercial sources by protection of the amino or hydroxyl group with a suitable protecting group, for example trityl, silyl, benzyl etc as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3rd Edition, John Wiley and Sons Inc. Formation of the diazepinone ring in the presence of a suitable base produces 17. Removal of protecting group or conversion to amine group from a precursor, such as a nitro group, followed by treatment with reagent 6 yield 5a. For example (Example 4), the hydroxyl group of 2-hydroxy ethylamine is protected as its MOM-ether (19). Selective displacement of 2'-chloro substituent of the pyridinecarboxamide ring with substituted ethylamine 19 produce 16a. Formation of the diazepinone ring in the presence of sodium hexamethyldisilazane affords 17a. MOM- is then removed to provide alcohol 18a. The hydroxyl group is then used for attaching the phosphonate group. The alcohol is first converted to carbonate by reacting with bis(4-nitrobenzyl)carbonate, subsequent treatment of the resulting carbonate with aminoethyl phosphonate 6.2 provides phosphonate 5a.1.

Scheme 5

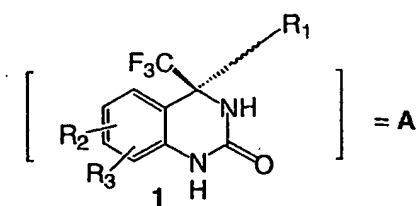


Example 5



Quinazolinone-like phosphonate NNRTI compounds

The present invention describes methods for the preparation of phosphonate analogs of quinazolinones shown in Figure 1 that are potential anti-HIV agents.

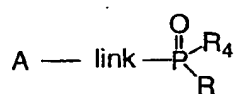


~~~~ = singl, double, triple bond

$R_1$  = substituted  $C_{3-5}$  alkyl,  $C_{3-5}$  cycloalkyl  
phenyl and heterocyclic, substituents  
are  $C_{1-4}$  alkyls, OH,  $C_{1-4}$ alkoxyl, halides,  
 $NH_2$ ,  $NHR_1'$ ,  $NR_1'R_1'$ ,  $NHCOR_1'$

$R_2$  = H, MeO, F, Cl

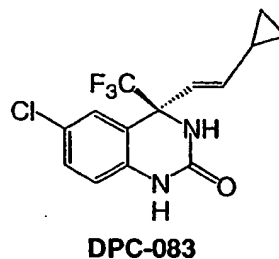
$R_3$  = H, F, Cl



link = linkage group

$R$  = OAr, O-heteroaryl, amino acid ester  
substituted OAr, O-heteroaryl

$R_4$  = amino acid ester,  $O-CH(R')-COOR''$

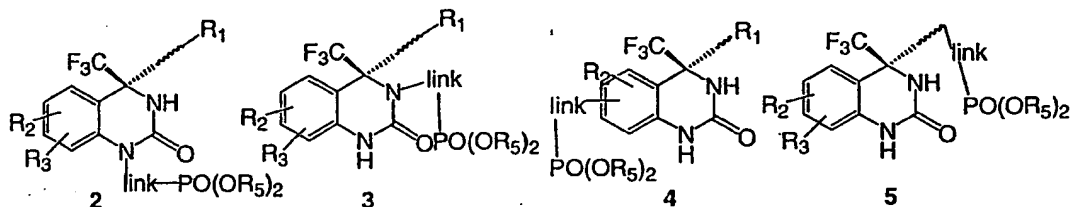


**Figure 1**

A link group includes a portion of the structure that links two substructures, one of which is quinoxalinone having the general formula shown above, the other is a phosphonate group bearing the appropriate R and  $R_4$  groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

Quinoxalinone class of compound, act as NNRTI, has demonstrated to inhibit HIV replication. DPC-083, one of representative analogs of this class of compounds, is in clinical phase II studies for treatment of HIV infection and AIDs. The present invention provides novel analogs of quinoxalinone class of compound. Such novel quinoxalinone analogs possess all the utilities of quinoxalinone and optionally provide cellular accumulation as set forth below.

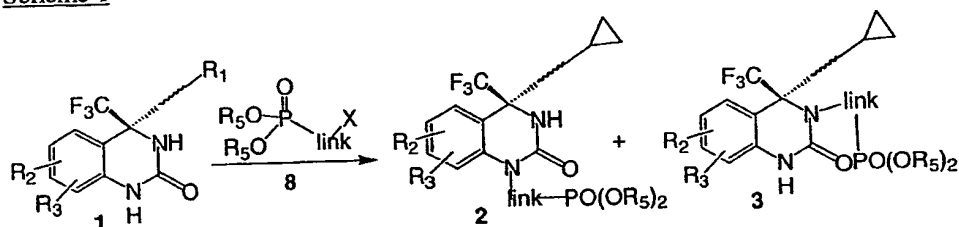
The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.



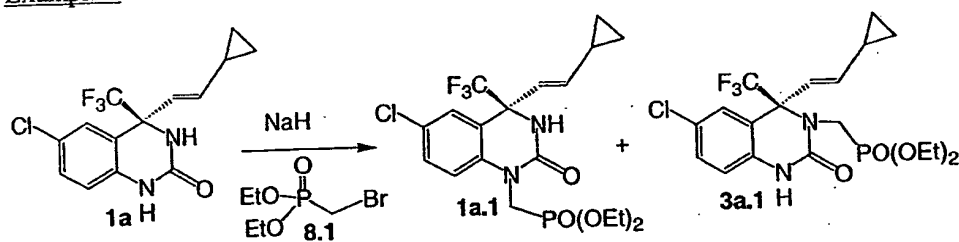
**Figure 2**

Preparation of phosphonate **2** is outlined in Scheme 1. Quinazolinone **1**, synthesized as described in Patent EP0530994, WO93/04047 and US Patent No. 6423718, is dissolved in suitable solvent such as, for example, DMF or other protic solvent is first treated with a suitable base to remove the urea proton, the product is then treated with 1 equivalent of a phosphonate reagent **8** bearing a leaving group such as, for example, bromine, mesyl, tosyl etc to give the alkylated product **2** and **3**. The phosphonates **2** and **3** are separated by chromatography. For example, **1** is dissolved in DMF, is treated with sodium hydride and 1 equivalent of bromomethyl phosphonic acid diethyl ester **8.1** prepared to give quinazolinone phosphonate **2** in which the linkage is a methylene group. Using the above procedure but employing different phosphonate reagents **8** in place of **8.1**, the corresponding products **2** and **3** are obtained bearing different linking group.

Scheme 1



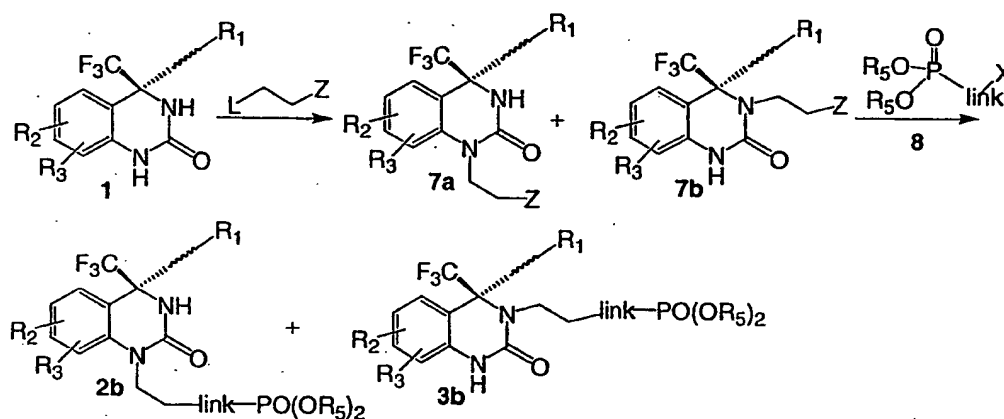
Example 1



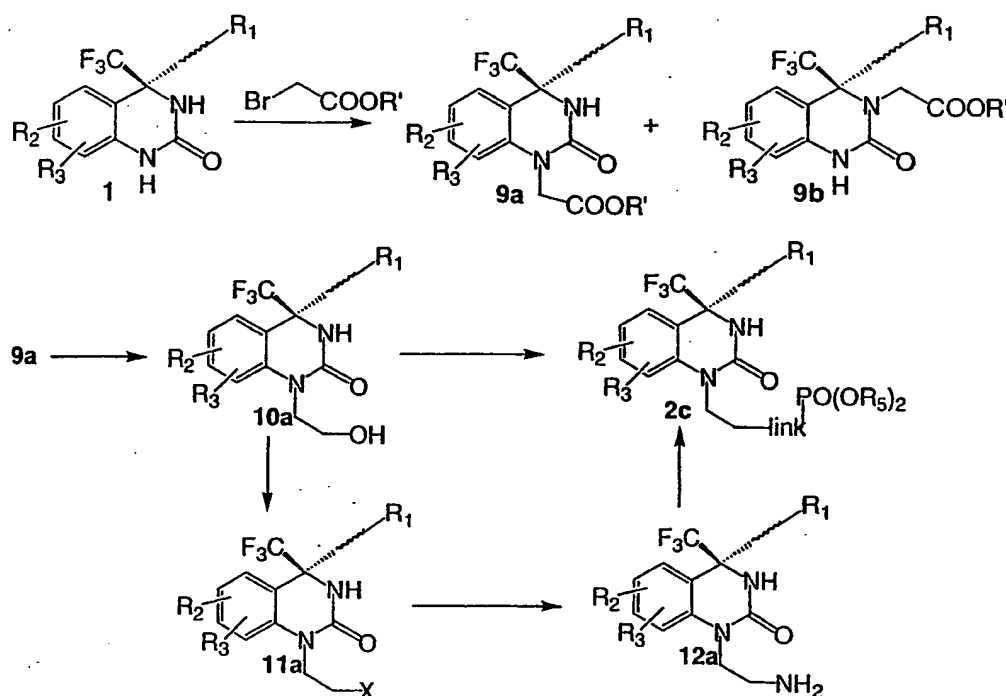
Scheme 2 shows the preparation of phosphonate analogs type **2** and **3** attached with an alternative way. Quinazolinone **1**, dissolved in a suitable solvent such as, for example, DMF or other protic solvents, is first treated with a suitable base to remove the urea proton, the product is then treated with 1 equivalent of reagent **B**, which bears a leaving group such as, for example, bromine, mesyl, tosyl etc, to give the alkylated product **7a** and **7b**. Compound **B** possesses a protected  $NH_2$  or  $OH$  group, or a precursor for them. The alkylated product **7a** and **7b** are separated by chromatography. Protecting group is then removed, and the resulting alcohol or amine then reacts with reagent **8** to afford **2b** and **3b** respectively.

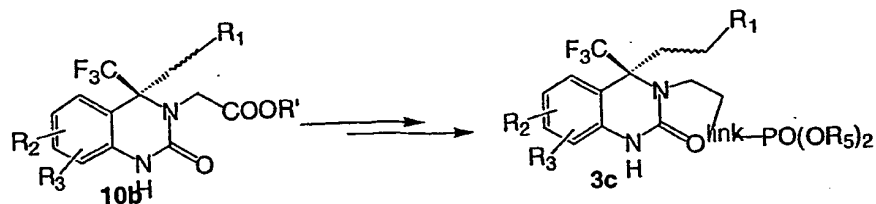
Alternatively (Scheme 3), alkylation of **1** with bromoacetate provides **9a** and **9b**, which are separated by chromatography. The ester group of **9** is reduced to alcohol to give **10**. The alcohol **11** is also transformed to amine **12** under conventional conditions, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. The hydroxyl group of **10** and amino group of **12** then serve as the attachment site for linking phosphonate to provide **2c**. Similarly, ester **10a** is converted to phosphonate **3c** following the procedures of transformation of **10** to **2c**.

Scheme 2



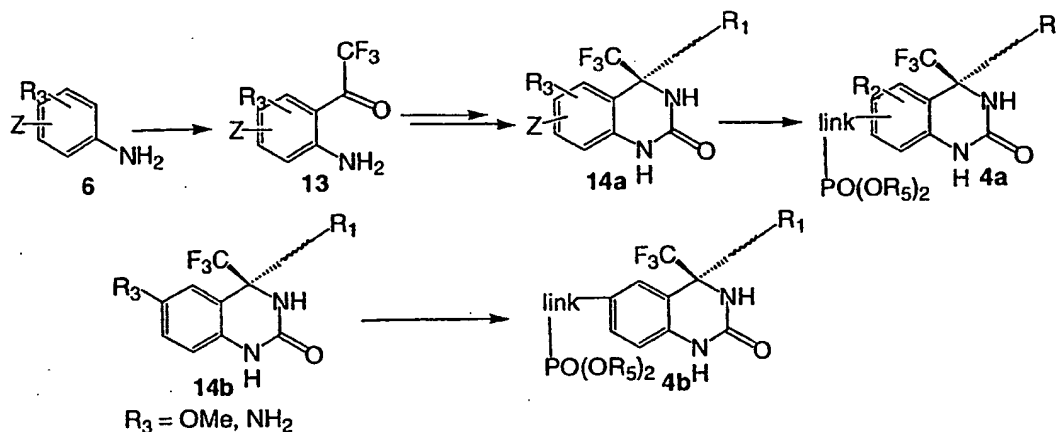
Scheme 3





Scheme 4 shows the preparation of quinazolinone-phosphonate conjugates type 4 in Figure 2. Substituted aniline 6 with a functional group Z, which is bearing a protected alcohol or amino group, or protected alcohol or amino alkyl, is converted to trifluoromethyl phenyl ketone 13, which is subsequently converted to quinoxalinone 14a, following the procedure described in US Patent No. 6423718. Deprotection of the protecting group, followed by reacting with reagents 8 under suitable conditions give the desired the phosphonate 4a. Quinoxaline 14b, prepared according to US Patent No. 6423718, is converted to phosphonate 4b by reacting with phosphonate reagent 8 directly ( $R_3 = NH_2$ ), or after deprotection ( $R_3 = OMe$ ) under the condition such as for example,  $BCl_3$ , many examples are described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. Synthesis of compound 6 is described in Scheme 5.

Scheme 4



Scheme 5 shows compounds **6** are obtained through modification of commercial available material 2-halo-5-nitroaniline, or 5-halo-2-nitroaniline (**6.0a**). The amino group of **6.0a** is first protected with a suitable protecting group, for example trityl, Cbz, or Boc etc as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. Reduction of the nitro group of **6.1a** with a reducing agent, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed, gives **6.1b**, which is then used in the transformation described in Scheme 4.

The amino group of **6.0a** is converted to hydroxyl group to give **6.2a** by established procedures, for example, diazotization followed by treatment with H<sub>2</sub>O/H<sub>2</sub>SO<sub>4</sub>, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. The hydroxyl group is then protected with a suitable protecting group, for example trityl ethers, silyl ethers, methoxy methyl ethers etc as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. The nitro group of the resulting compound is then reduced with the above mentioned methods to give **6.2b**, which is then used in the transformation described in Scheme 4.

The hydroxyl or amino alkyls are obtained using the following methods. The amino group of **6.0a** is converted to nitrile **6.3a** with the known method, for example diazotization followed by treatment with cuprous cyanide, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. The nitrile group is then selectively reduced with a reducing agent, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed, to give amine **6.3b**. With the mentioned methods above, the amino group is protected and nitro group is reduced respectively to give **6.3c**. Alternatively, the nitrile **6.3a** is converted to acid **6.4a** and the acid is subsequently reduced to alcohol to give **6.4b** using the examples described in R. C. Larock,

Comprehensive Organic Transformation, John Wiley & Sons, 2<sup>nd</sup> Ed. Similarly, protection of hydroxyl group followed by reduction of nitro to amine gives **6.4c**. Compound **6.3c** and **6.4c** are used in Scheme 4 respectively.

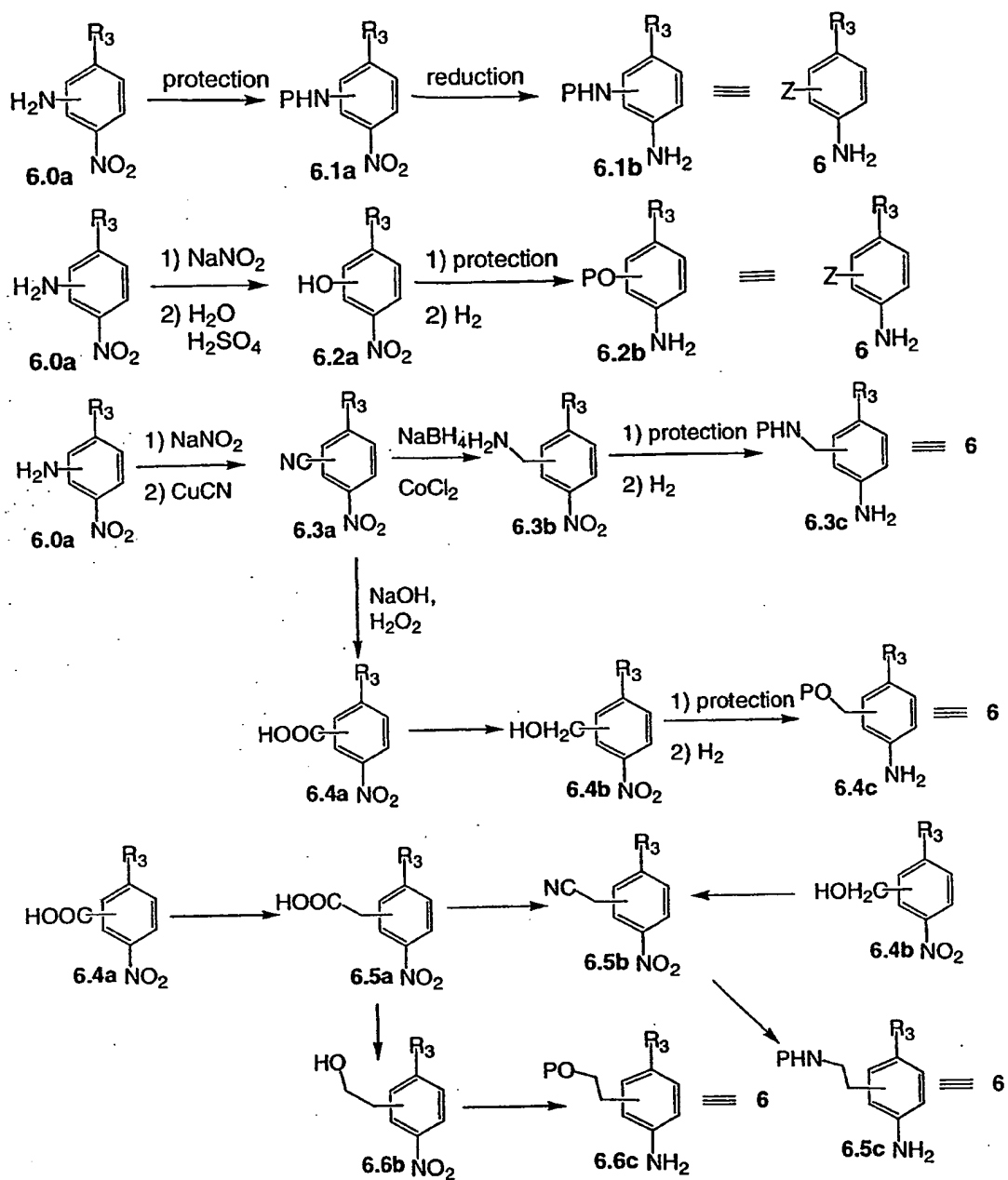
The homologated hydroxyl or amino alkyls are obtained using the following methods (Scheme 3). The acid **6.4a** are extended to acid **6.5a**, which is transformed to nitrile **6.5b**, these two transformation are described in R. C. Larock, Comprehensive Organic Transformation, John Wiley & Sons, 2<sup>nd</sup> Ed, Nitrile **6.5b** is converted to aniline **6.5c** using the similar methods described above. Alternatively, nitrile **6.5b** is obtained by first convert benzyl alcohol **6.4b** to benzyl halide, then treated with CN<sup>-</sup> nucleophile. Reduction of acid **6.5a** provided alcohol **6.6b**, which is protected using the protecting groups described above to give the required aniline **6.6c**. Compound **6.5c** and **6.6c** are used in Scheme 4 respectively.

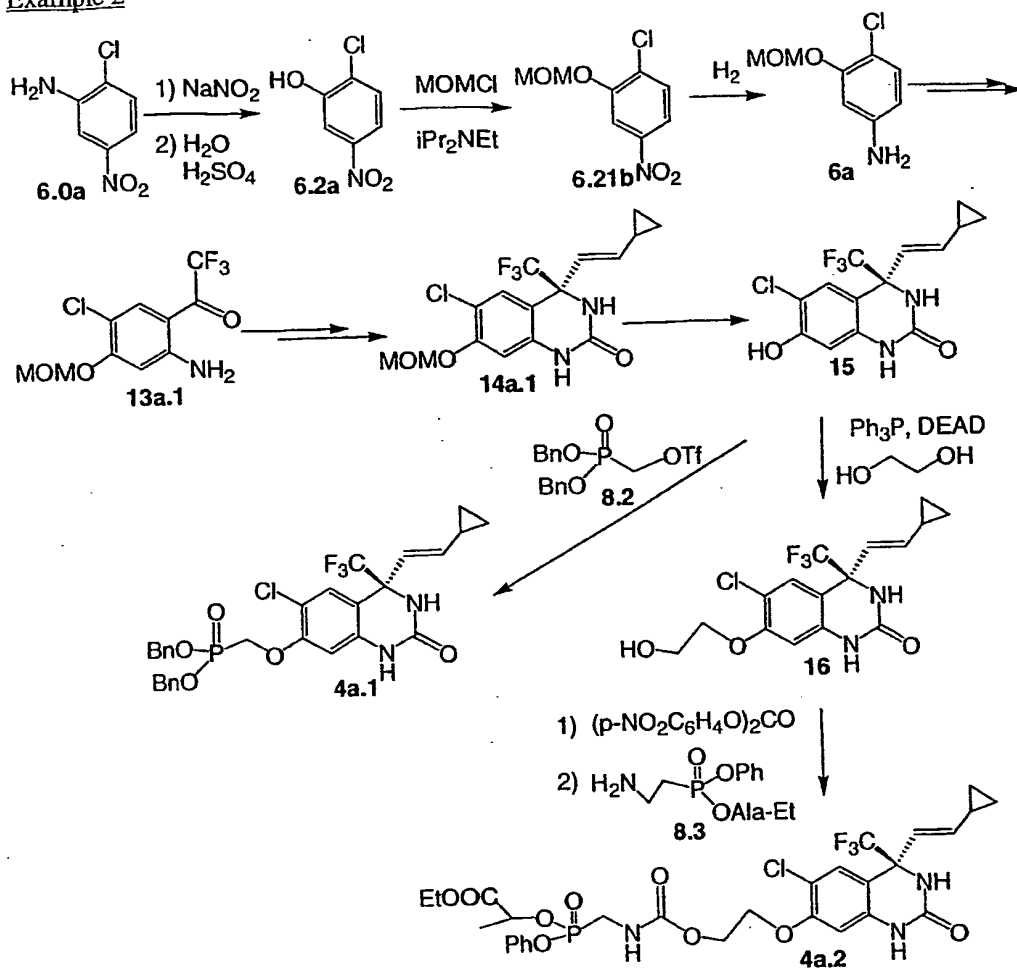
For example aniline **6.0a** (Example 2) is treated with NaNO<sub>2</sub> in the presence of acid at 0°C, then the resulting mixture was heated in H<sub>2</sub>O to give phenol **6.2a**. The hydroxyl group is then protected as methoxyl methyl ether by treating phenol **6.2a** with MOMCl in the presence of Hunig's base to yield **6.21b**. Hydrogenation of nitrobenzene affords aniline **6a**. Aniline **6a** is converted to phenyl trifluoromethyl ketone **13a.1**, which is subsequently transformed to quinazolinone analog **14a.1**, using the method described in US Patent No. 6423718. Deprotection of the MOM-ether with trifluoroacetic acid provides phenol **15**. Treatment of **15**, in acetonitrile, with triflate methyl phosphonic acid dibenzyl ester **8.2** in the presence of Cs<sub>2</sub>CO<sub>3</sub> gives **4a.1**. Alternatively, reaction of phenol **15** with ethylenediol under the Mitsunobu condition produces **16**. Hydroxyl group of **16** as activated as carbamate, subsequent treatment with amino methyl phosphonate **8.3** affords phosphonate analog **4a.2**.

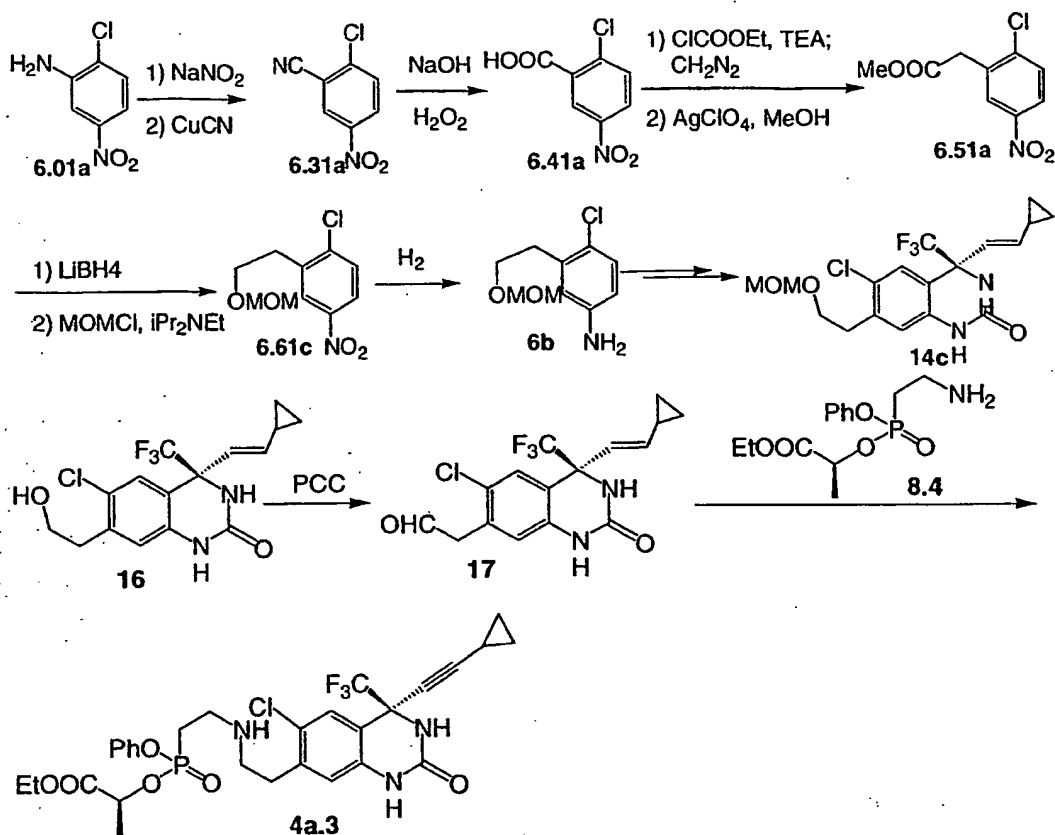
Example 3 shows 2-chloro-5-nitro aniline **6.0b** transformed to nitrile **6.31a** by reacting with NaNO<sub>2</sub> and then CuCN subsequently. Hydrolysis of nitrile **6.31a** gives acid **6.41a**. Treatment of **6.41a** with ClCOOEt in the presence of base at 0°C followed by CH<sub>2</sub>N<sub>2</sub> provides diazoketone, which is converted to methyl ester **6.51a** upon treating with silver perchlorate in methanol. The ester group is then reduced to give alcohol, which is protected as MOM-ether to provide **6.61c**. The nitro group is then reduced to amine to afford **6b**. Aniline **6b** is converted to quinazolinone analog **14** using the method described in US Patent No. 6423718. Deprotection of the MOM-ether with trifluoroacetic acid provide alcohol **16**. The aldehyde **17** is obtained by oxidation of alcohol. Reductive amination of **17** with amino ethyl phosphonate **8.4** afford analog **4a.3**.

#### Scheme 5

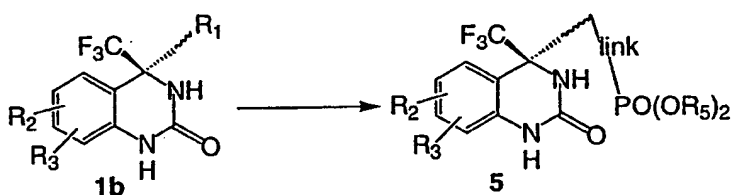




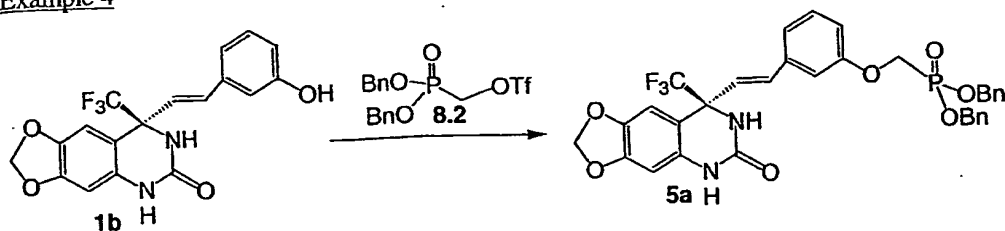
Example 2

**Example 3**

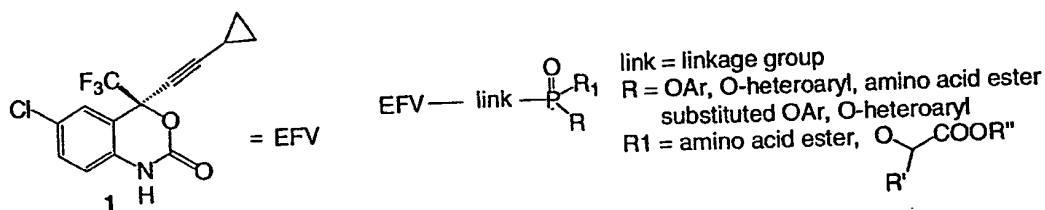
Preparation of phosphonate analog type 5 from quinoxalinone 1 is outlined in Scheme 6. Quinoxalinone 1, which  $\text{R}_1$  contains OH, or  $\text{NH}_2$  or  $\text{NHR}_1$ , as the attachment site for connecting phosphonate, reacts with reagent 8 under suitable conditions to provide phosphonate analog 5. For example (Example 4), Quinoxalinone **1b.1**, obtained as described in US Patent No. 6423718, is treated with phosphonate reagents **8.2** in the presence of  $\text{Cs}_2\text{CO}_3$ , give phosphonate **5a**.

**Scheme 3**

$\text{R}_1$ : defined as above but contains OH,  $\text{NH}_2$

Example 4Efavirenz-like phosphonate NNRTI compounds

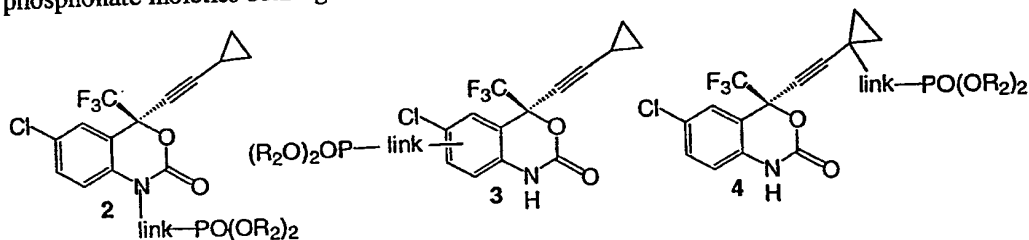
The present invention includes efavirenz-like phosphonate NNRTI compounds and methods for the preparation of efavirenz phosphonate analogs shown in Figure 1.

Figure 1.

A link group includes a portion of the structure that links two substructures, one of which is efavirenz having the general formula shown above, the other is a phosphonate group bearing the appropriate R and R<sub>1</sub> groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

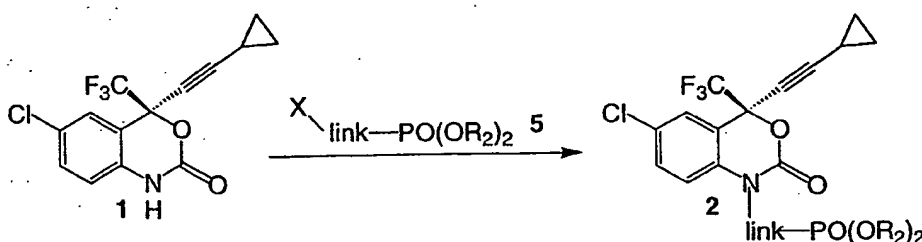
Efavirenz and its analogs have demonstrated therapeutic activity against HIV replication, and efavirenz is currently used in clinical for treatment of HIV infection and AIDS. The present invention provides novel analogs of efavirenz. Such novel efavirenz analogs possess all the utilities of efavirenz and optionally provide cellular accumulation as set forth below.

The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.

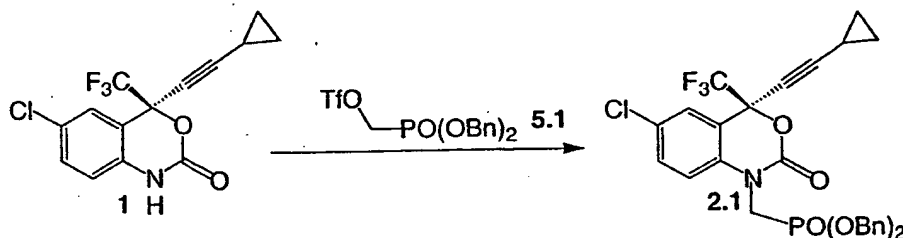
Figure 2

Compound 1 can be synthesized as described in US Patent No. 5519021. Preparation of compound 2 from efavirenz 1 is outlined in Scheme 1. Efavirenz 1 is dissolved in suitable solvent such as, for example, DMF or other protic solvent, and treated with the phosphonate reagent 5 in the presence of a suitable organic or inorganic base. For example, 1 is dissolved in DMF, is treated with sodium hydride and 1 equivalent of triflate methyl phosphonic acid dibenzyl ester 5.1 prepared to give EFV phosphonate 2 in which the linkage is a methylene group. Using the above procedure but employing different phosphonate reagents 5 in place of 5.1, the corresponding products 2 are obtained bearing different linking group.

Scheme 1.

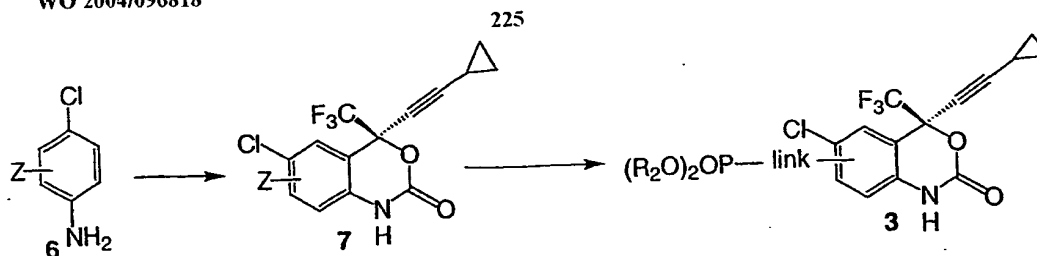


Example 1



Scheme 2 shows the preparation of EFV-phosphonate conjugates compounds 3 in Figure 2. *p*-Chloro aniline with functional group Z, which bears a protected alcohol or amino group, or protected alcohol or amino alkyl, is converted to compound 7 following the procedure described in US Patent No. 5519021. Deprotection of the protecting group, followed by reacting with reagent 5 in the above mentioned conditions give the desired the compound 3. As shown in Scheme 3, compounds 6 are obtained through modification of commercial available material 2-chloro-5-nitroaniline, or 5-chloro-2-nitroaniline (6.0a).

Scheme 2



The amino group of **6.0a** is first protected with a suitable protecting group (Scheme 3), for example trityl, Cbz, or Boc etc as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. Reduction of the nitro group in **6.1a** with a reducing agent, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed, give **6.1b**, which is then used in the transformation described in Scheme 2.

Alternatively, the amino group of **6.0a** is converted to hydroxyl group to give **6.2a** by established procedures, for example, diazotization followed by treatment with  $H_2O/H_2SO_4$ , many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. The hydroxyl group is then protected with a suitable protecting group, for example trityl ethers, silyl ethers, methoxy methyl ethers etc as described in Greene and Wuts, *Protecting Groups in Organic Synthesis*, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. The nitro group of the resulting compound is then reduced with the above mentioned methods to give **6.2b**, which is then used in the transformation described in Scheme 2.

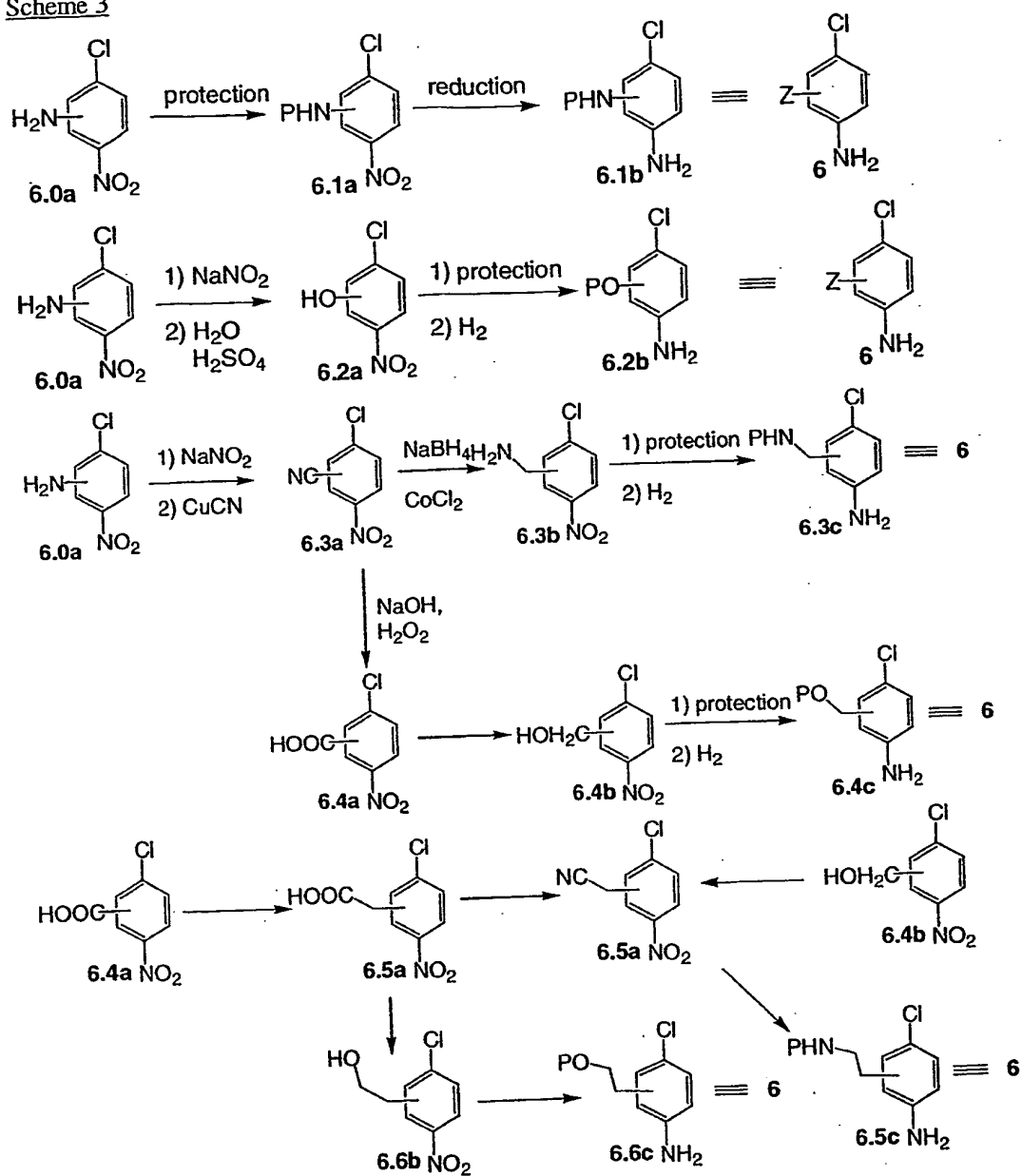
The hydroxyl or amino alkyls are obtained using the following methods. The amino group in **6.0a** is converted to nitrile **6.3a** with the known method, for example diazotization followed by treatment with cuprous cyanide, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. The nitrile group is then selectively reduced with a reducing agent, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed, to give amine **6.3b**. With the mentioned methods above, the amino group is protected and nitro group is reduced respectively to give **6.3c**. In addition, the nitrile **6.3a** is converted to acid **6.4a** and the acid is subsequently reduced to alcohol to give **6.4b**, and the reduction of nitro to amine give **6.4c**, using the methods described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. Both **6.3c** and **6.4c** used in the transformation described in Scheme 2. The homologated hydroxyl or amino alkyls are obtained using the following methods (Scheme 3). The acid **6.4a** are extended to acid **6.5a**, which is transformed to nitrile **6.5b**, these two transformation are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed, Nitrile **6.5b** is converted to aniline **6.5c** using the similar methods

described above. Alternatively, nitrile **6.5b** is obtained by first convert benzyl alcohol **6.4b** to benzyl halide, then treated with CN<sup>-</sup> nucleophile. Reduction of acid **6.5a** provided alcohol **6.6b**, which is protected using the protecting groups described above to give the required aniline **6.6c**. Both **6.5c** and **6.6c** used in the transformation described in Scheme 2.

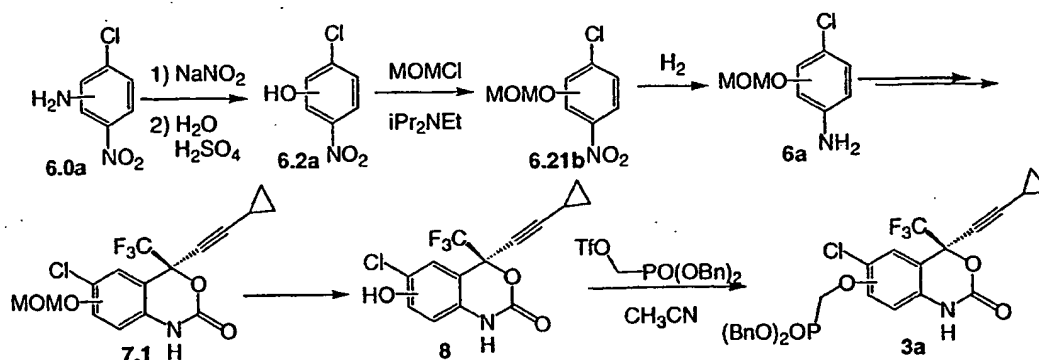
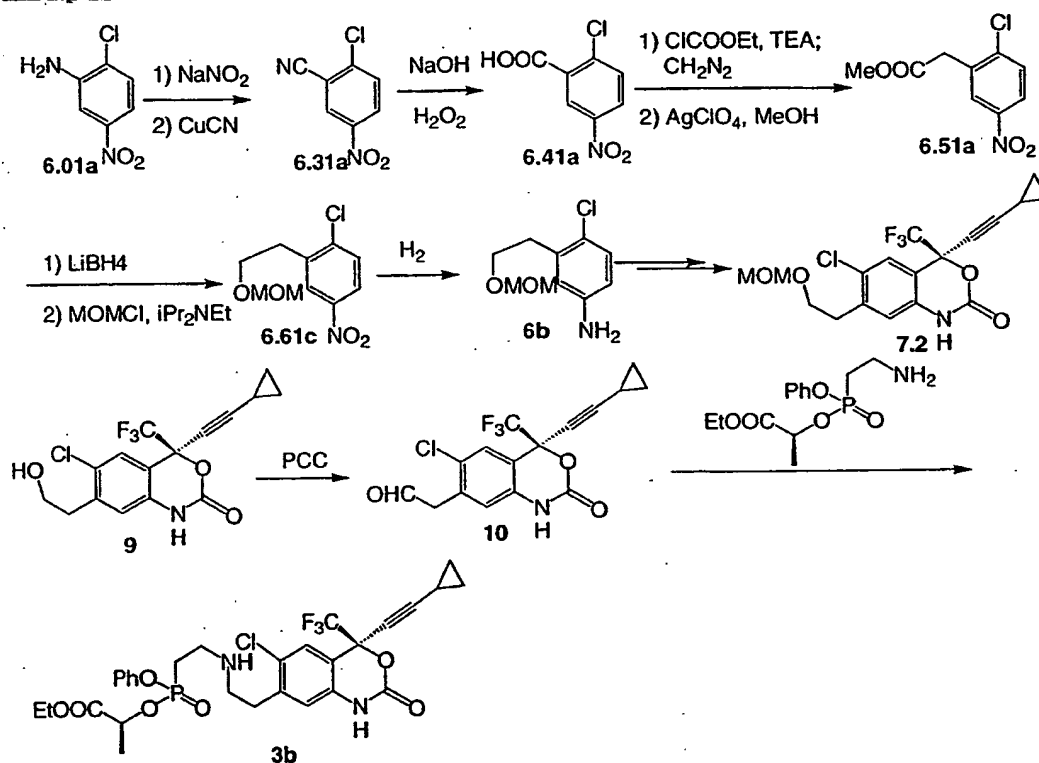
For example aniline **6.0a** (Example 2) is treated with NaNO<sub>2</sub> in the presence of acid at 0°C, then the resulting mixture was heated in H<sub>2</sub>O to give phenol **6.2a**. The hydroxyl group is then protected as methoxyl methyl ether by treating phenol **6.2a** with MOMCl in the presence of Hunig's base to yield **6.21b**. Hydrogenation of nitrobenzene affords aniline **6.2a**. Aniline **6a** is converted to efavirenz analog **7.1**. Deprotection of the MOM-ether with trifluoroacetic acid provides phenol **8**. Treatment of **8** in acetonitrile with (trifluorosulfonylmethyl)-phosphonic acid dibenzyl ester **5.1** in the presence of Cs<sub>2</sub>CO<sub>3</sub> gives **3a**.

In Example 3, 2-chloro-5-nitro aniline **6.0b** is transformed to nitrile **6.31a** by reacting with NaNO<sub>2</sub> and then CuCN subsequently. Hydrolysis of nitrile **6.31a** gives acid **6.41a**. Treatment of **6.41a** with ClCOOEt in the presence of base at 0°C followed by CH<sub>2</sub>N<sub>2</sub> provides diazoketone, which is converted to methyl ester **6.51a** upon treating with silver perchlorate in methanol. The ester group is then reduced to give alcohol, which is protected as MOM-ether to provide **6.61c**. The nitro group is then reduced to amine to afford **6b**. Aniline **6a** is converted to efavirenz analog **7.1**. Deprotection of the MOM-ether with trifluoroacetic acid provides phenol **9**. The aldehyde **10** is obtained by oxidation of alcohol. Reductive amination of **10** with agent **5.2** affords analog **3b**.

Scheme 3



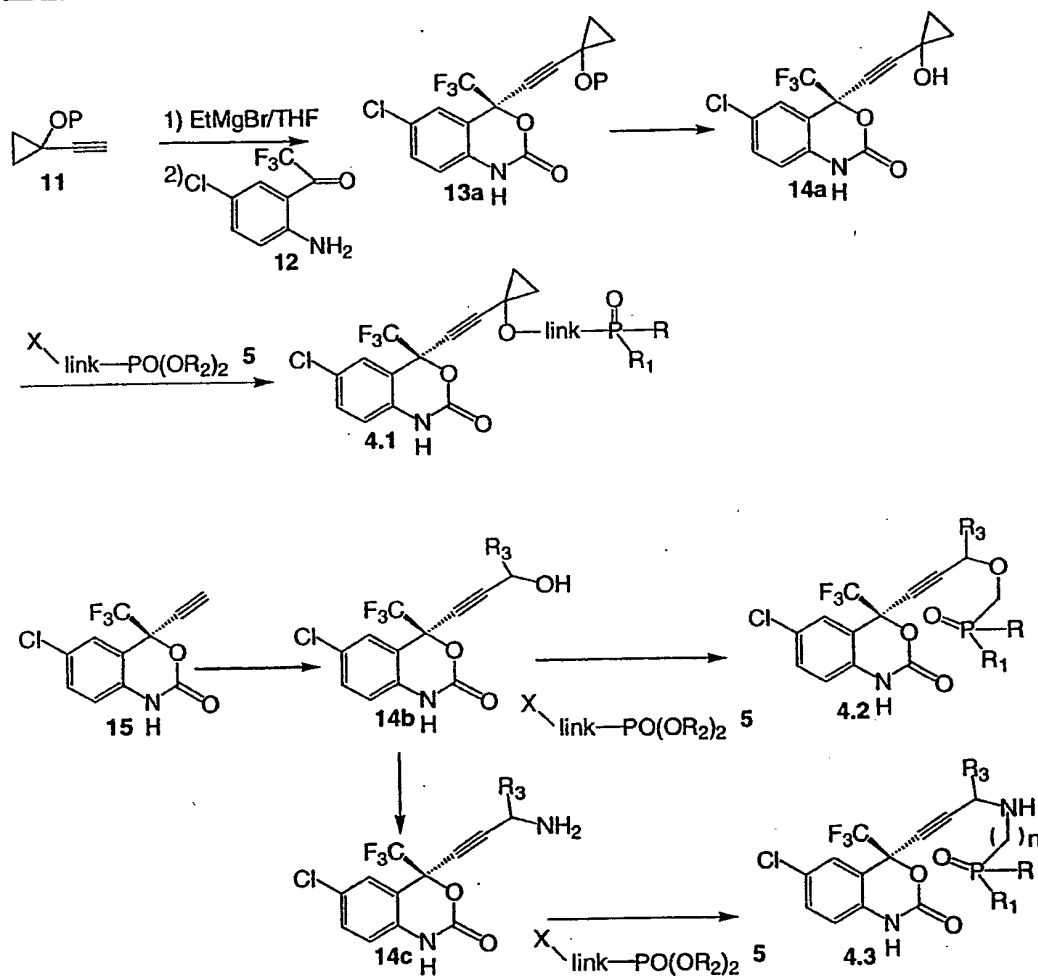


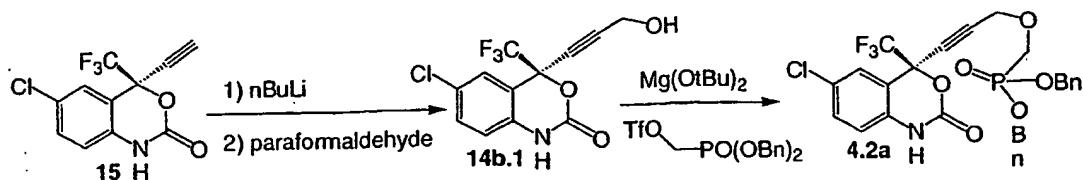
Example 2Example 3

Preparation of compound 2 from efavirenz 1 is outlined in Scheme 4. Compound 12, obtained as described in US Patent No. 5519021, reacting with Grignard reagent, generated from protected acetylene 11 following the procedure described in US Patent No. 5519021, gives compound 13a. The hydroxyl group in 11 is protected as its silyl ether, trityl ether etc. Removal of the protecting group of 13a yields alcohol 14a. Alkylation of 14a with agent 5 affords phosphonate 4.1. Alternatively, compound 15, obtained as described in US Patent No.

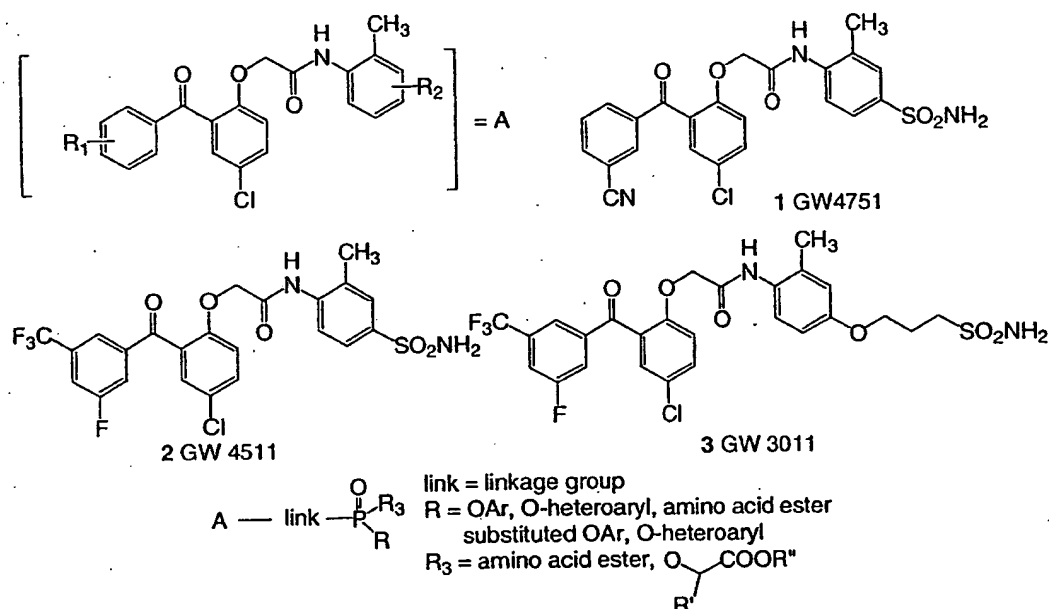
5519021, reacts with aldehyde or ketone to give alcohol **14b**, which is converted to analog **4b** using the conditions described above. Amine **14c** is obtained from alcohol **14b** under the standard conditions. Amine **14c** is converted to phosphonate **4c** either by reacting with agent **5** or reductive amination with a phosphonate reagents containing an aldehyde group. For example, treatment of compound **14** with *n*-BuLi followed by paraformaldehyde gives alcohol **14b.1**. Treatment of alcohol **14b.1** with  $\text{Mg}(\text{OtBu})_2$  followed by phosphonate provides phosphonate **4.2b**.

Scheme 4



**Example 4****Benzophenone-like phosphonate NNRTI compounds**

The present invention describes methods for the preparation of phosphonate analogs of benzophenone class of HIV inhibiting pyrimidines shown in Figure 1 that are potential anti-HIV agents.



$R_1$  = halide,  $CF_3$ , CN,  $NO_2$ ,  $C_{1-6}$  alkyl,  $OR^1$ ,  $NHR^1$ ,  $NHR^1R^2$ , where  $R^1$  and  $R^2$  are  $C_{1-6}$  alkyl  
 $R_2$  = OH,  $OR^1$ ,  $NHR^1$ ,  $NHR^1R^2$ ,  $SO_2NH_2$ ,  $SO_2NHR^1$ ,  $SONR^1R^2$ ,  $CONH_2$ ,  $CONHR^1$ ,  $OR^3$   
 where  $R^3$  is H or  $R_1$

**Figure 1**

A link group includes a portion of the structure that links two substructures, one of which is benzophenone class of HIV inhibiting agents having the general formula shown above, the other is a phosphonate group bearing the appropriate R and  $R_3$  groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

Benzophenone class of compounds has shown to be inhibitors of HIV RT. The present invention provides novel analogs of benzophenone class of compound. Such novel benzophenone analogs possess all the utilities of benzophenone and optionally provide cellular accumulation as set forth below.

The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.

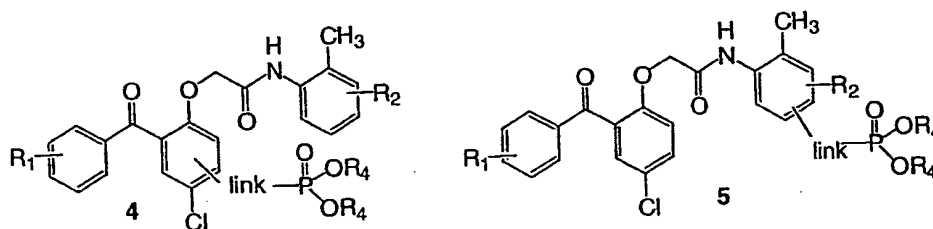


Figure 2

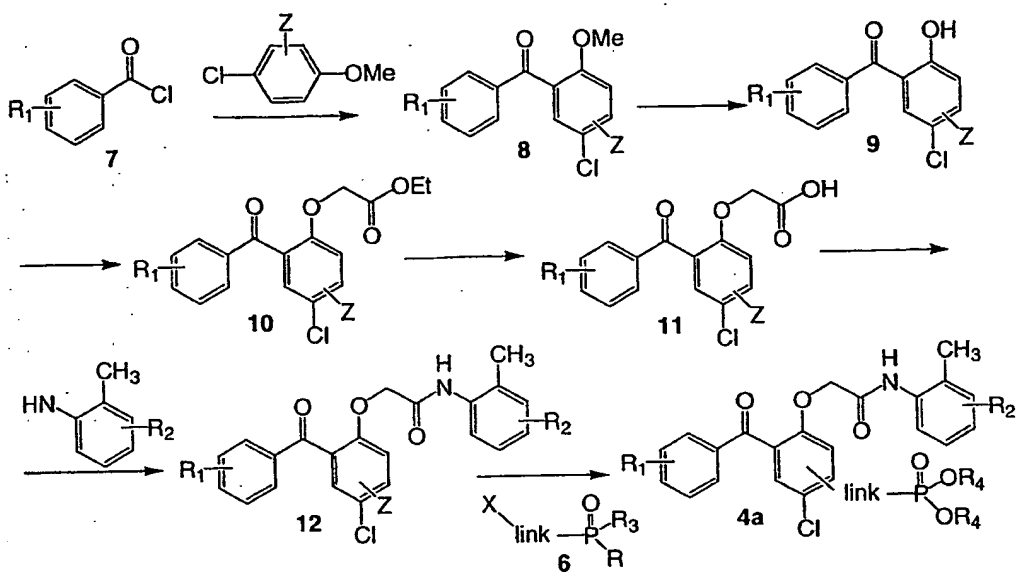
Preparation of phosphonate analog 4 is outlined in Scheme 1. Benzophenone 8 is obtained from Freidel-Crafts reaction of substituted benzoyl chloride 7 and 4-chloro-phenol methyl ether which bearing a protected amine or hydroxyl group Z. Phenol ether is obtained by selective protection of commercially available 4-chlorophenol substituted with amino- or hydroxyl group. Benzoyl chloride is obtained either from commercial sources or prepared from commercial available benzoic acid. Benzophenone 8 is also obtained from oxidation of the corresponding alcohol, which in turn is obtained from the reaction of benzaldehyde and anion. Removal of methyl provides phenol 9. Alkylation of phenol with bromoacetate such as ethyl bromoacetate affords ester 10. The ester is then converted to acid. Formation of amide 12 from acid 11 and aniline 10 is achieved following the standard amide formation methods, many examples are described in R. C. Larock, Comprehensive Organic Transformation, John Wiley & Sons, 2<sup>nd</sup> Ed. Removal of the protecting group of Z followed by reacting with reagent 6 affords phosphonate analog 4a.

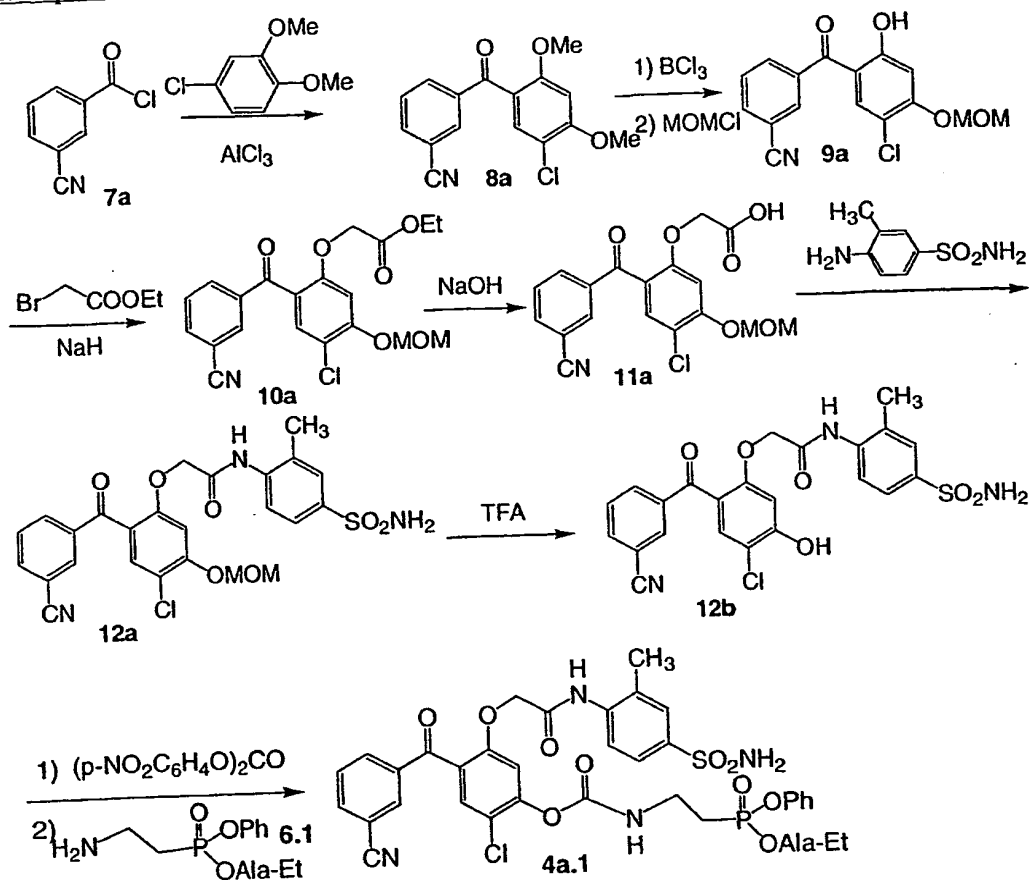
For example (Example 1), commercially available 3-cyanobenzoyl chloride is treated with trichloroaluminum followed by 3,4-dimethoxy chlorobenzene to give benzophenone 8a. Treatment of 8 with BCl<sub>3</sub> removes the methyl to give diphenol, which is selectively protected as its mono MOM-ether to give 9a. Alkylation of phenol 9a with ethyl bromoacetate gives ester 10a. Hydrolysis of the ester affords acid 11a. Coupling of the acid 11a with aniline produces 12a. The MOM- group is then removed to yield phenol 12b. Phenol is then

activated as its 4-nitro-phenyl carbonate by reacting with bis(4-nitro-phenyl)carbonate, which is subsequently treated with aminoethyl phosphonate to give 4a.1.

Alternatively (Scheme 2), amine 10 is transformed to phenol 11 as described in, the hydroxyl group is then serves as the linking site for a suitable phosphonate group.

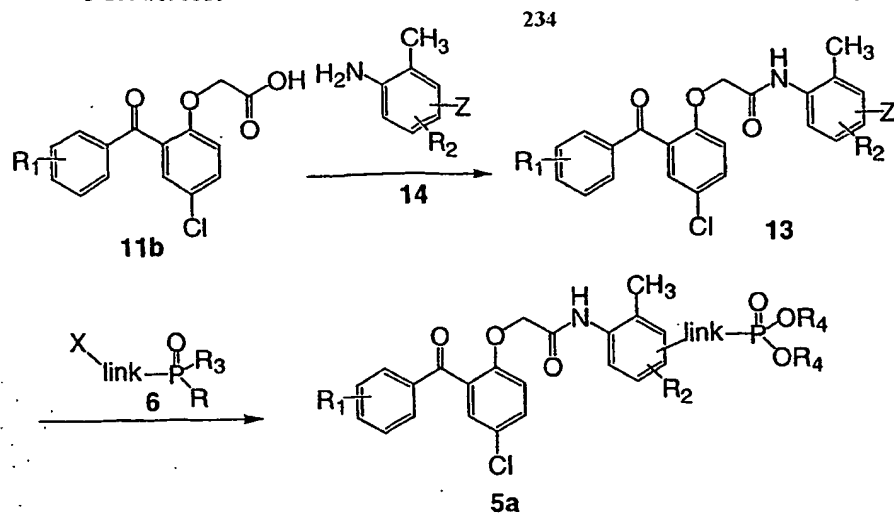
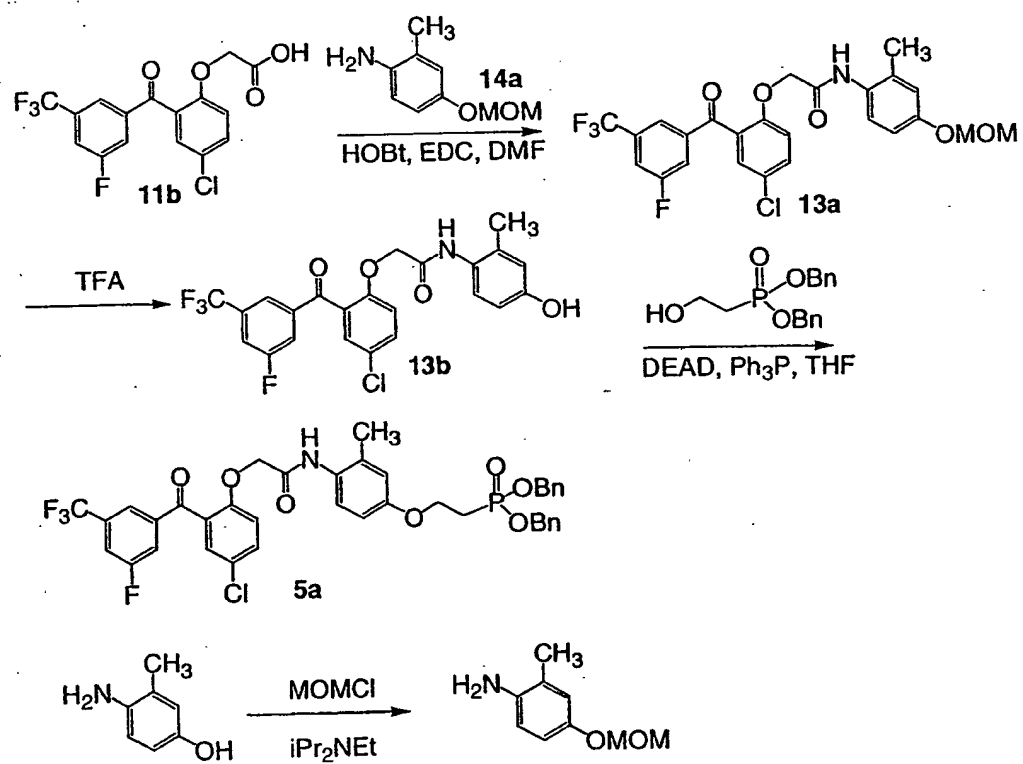
Scheme 1



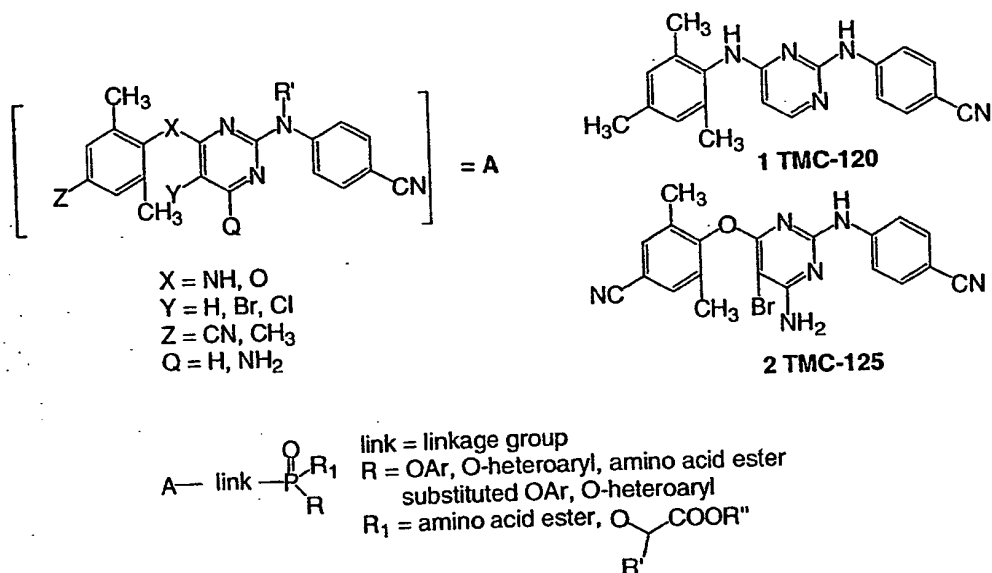
**Example 1**

Scheme 2 shows the preparation of phosphonate analog type 5. Benzophenone **11b** reacts with aniline **14**, bearing a protect hydroxyl or amino group, gives amide **13**. Formation of amide **13** from acid **11b** and aniline **14** is achieved following the standard amide formation methods, many examples are described in R. C. Larock, *Comprehensive Organic Transformation*, John Wiley & Sons, 2<sup>nd</sup> Ed. Removal of the protecting group of **Z** followed by reacting with reagent **6** affords phosphonate analog **5a**. For example (Example 2), acid **11b** couples with aniline **14** provides amide **13a**. The MOM-group is then deprotected with TFA to afford phenol **13b**, which is then coupled with hydroxy ethyl phosphonic acid dibenzyl ester to afford phosphonate **5a**. Protected aniline **14a** is obtained by treating the commercially available 4-amino-m-cresol with MOMCl in the presence of base, for example Hunig's base.

**Scheme 2**

Example 2Pyrimidine-like phosphonate NNRTI compounds

The present invention includes Pyrimidine-like phosphonate NNRTI compounds. The present invention also includes methods for the preparation of phosphonate analogs of TMC-125 and TMC-120 class of HIV inhibiting pyrimidines as shown in Figure 1 which are potential anti-HIV agents.



**Figure 1**

A link group includes a portion of the structure that links two substructures, one of which is TMC-120 and TMC-125 class of pyrimidines having the general formula shown above, the other is a phosphonate group bearing the appropriate R and R<sub>1</sub> groups. The link has at least one uninterrupted chain of atoms other than hydrogen.

TMC-125 and TMC-120 class of pyrimidines have demonstrated to be potent in inhibition of HIV replication. Both TMC-125 and TMC-120 are currently in clinical phase II studies for treatment of HIV infection and AIDs. The present invention provides novel analogs of TMC-120 and TMC-125 class of compound. Such novel TMC-120 and TMC-125 class analogs possess all the utilities of TMC-120 and TMC-125 class and optionally provide cellular accumulation as set forth below.

The intermediate phosphonate esters required for conversion into the prodrug phosphonate moieties bearing amino acid, or lactate esters are shown in Figure 2.



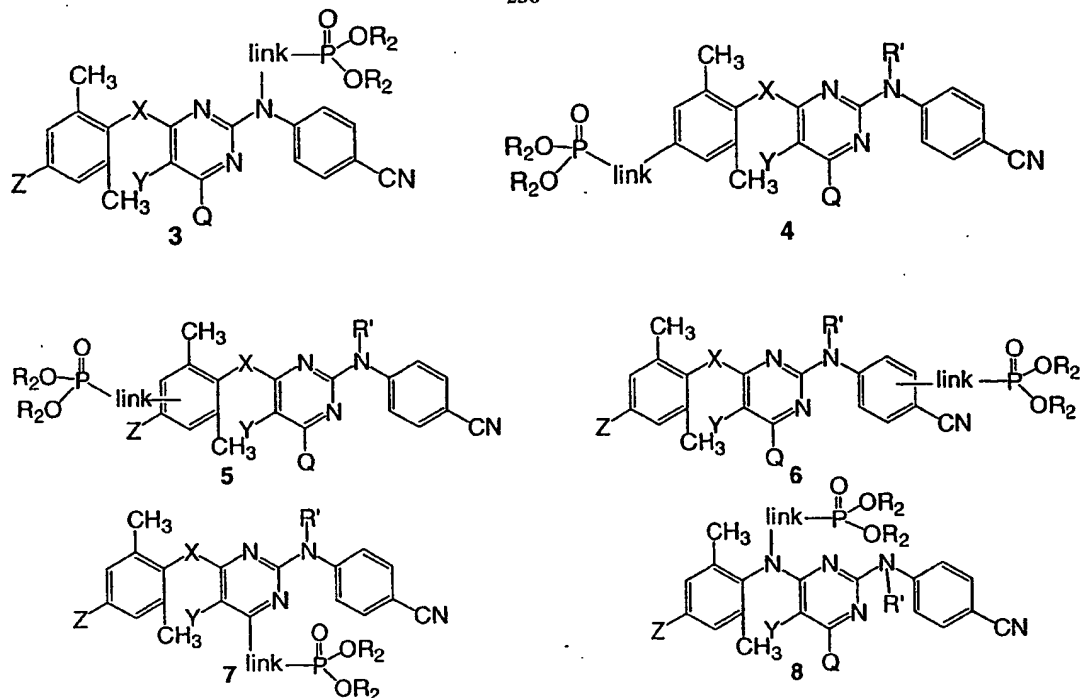
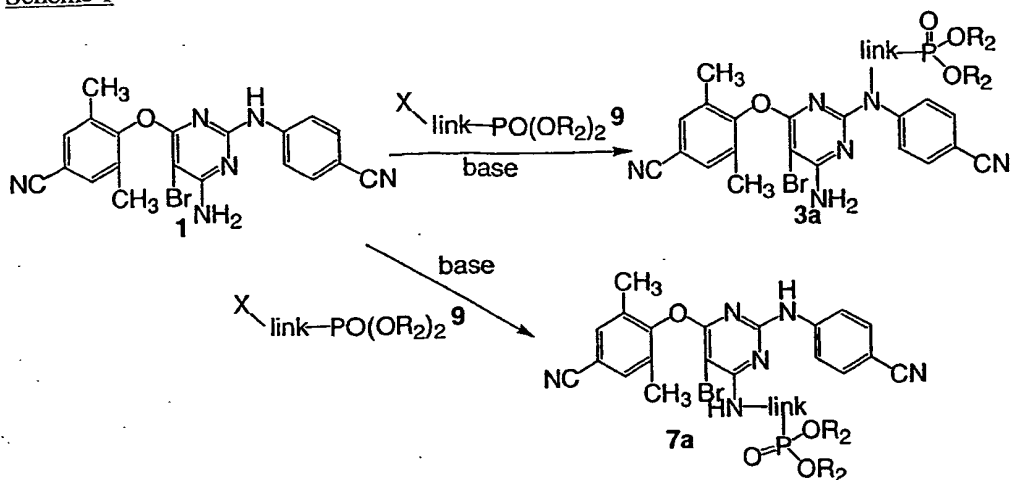


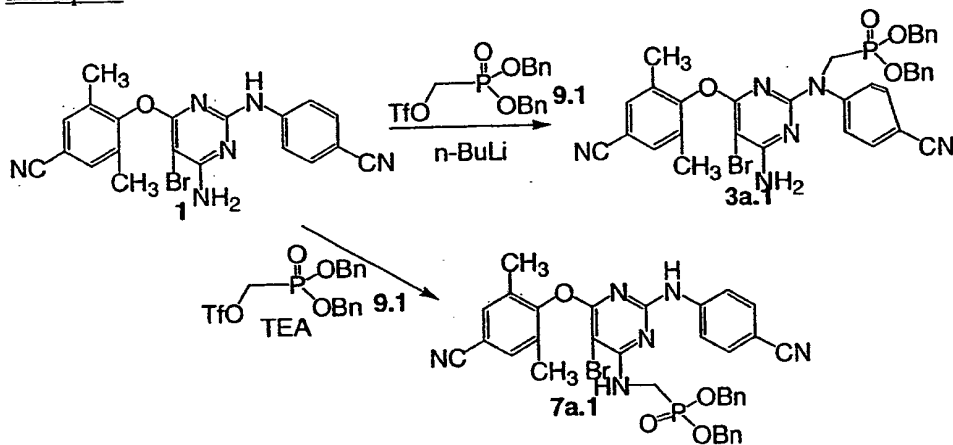
Figure 2

Compounds 1 and 2 can be synthesized as described in US Patent No. 6197779 and WO 0027825. Preparation of phosphonate analog 3 and 7 is outlined in Scheme 1. TMC-125 1 is dissolved in suitable solvent such as, for example, DMF or other protic solvent, and treated with the phosphonate reagent 9, bearing a leaving group, such as, for example, bromine, mesyl, tosyl, or trifluoromethanesulfonyl in the presence of a suitable organic or inorganic base, either 3a or 7a is obtained as the major product depending on the base. For example, 1 was dissolved in DMF, is treated with n-butyl lithium and 1 equivalent of triflate methyl phosphonic acid dibenzyl ester 9.1 prepared to give phosphonate 3a.1 as the major product. Alternatively, treatment of 1 with 9.1 in acetonitrile in the presence of triethylamine provides 7a.1 as the major product. The above procedure provides phosphonate analog 3 in which the linkage is a methylene group. Using the above procedure but employing different phosphonate reagents 9 in place of 9.1, the corresponding products 3 and 7 are obtained bearing different linking group.

Scheme 1

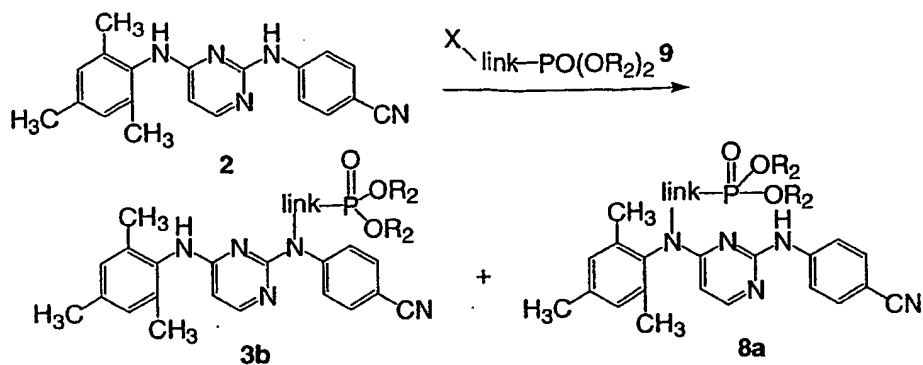
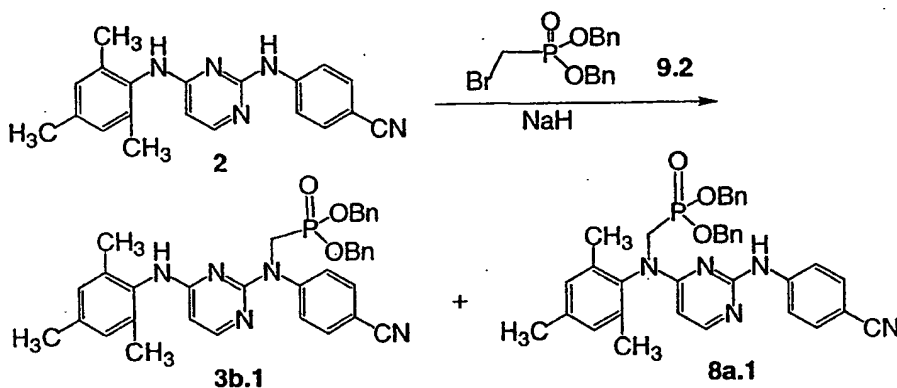


Example 1



Scheme 2 shows the preparation of phosphonate conjugates compounds type 3 and 8 in Figure 2. TMC-120 2 is treated with base, and subsequently treated with phosphonate reagent 9 bearing a leaving group, such as, for example, bromine, mesyl, tosyl, or trifluoromethanesulfonyl. The alkylated products are then separated by chromatography. For example (Example 2), treatment of TMC-120 2 with NaH in DMF, followed by bromomethyl phosphonic acid dibenzyl ester 9.2 gives phosphonate 3b.1 and 8a.1. The mixture of phosphonates 3b.1 and 8a.1 is separated by chromatography to give pure 3b.1 and 8a.1 respectively.

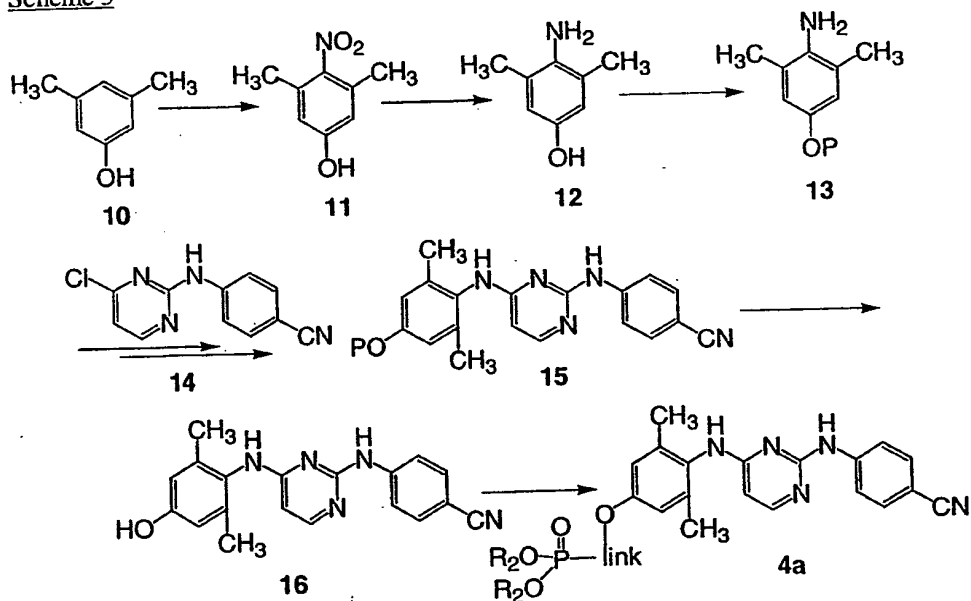
Scheme 2

**Example 2**

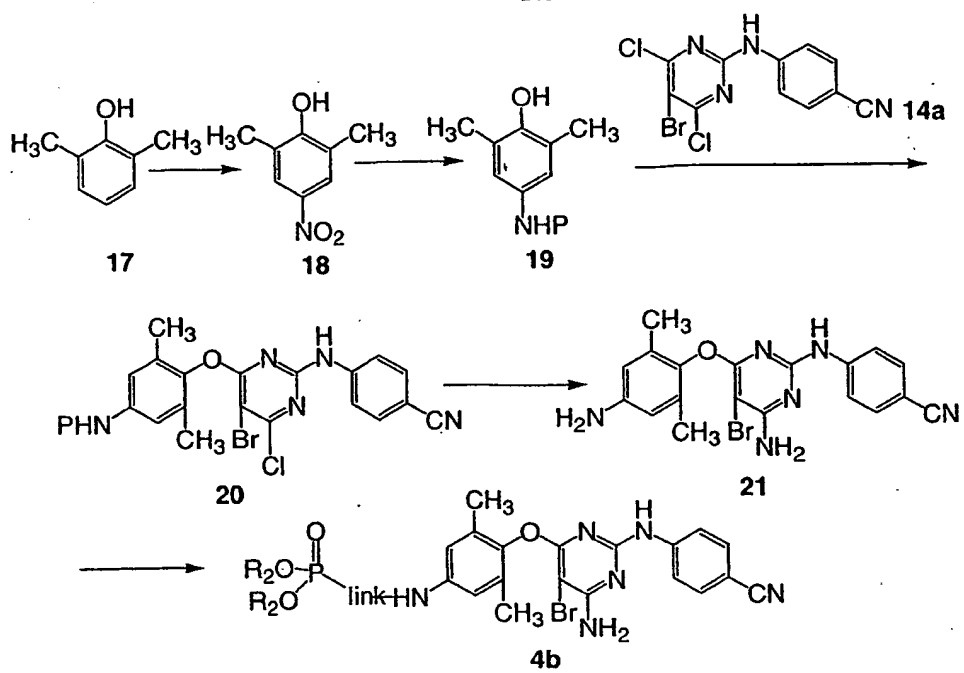
Preparation of phosphonate analogs type **4** in Figure 2 is shown in Scheme 3, 4 and 5. Nitration of commercially available 3,5-dimethyl phenol **10** gives **11**, subsequent reduction of the resulting nitrobenzene **11** provide **12**, many examples are described in R. C. Larock, Comprehensive Organic Transformation, John Wiley & Sons, 2<sup>nd</sup> Ed. The hydroxyl group of phenol **12** is protected with a suitable protecting group, for example trityl, silyl, benzyl or MOM- etc to give **13** as described in Greene and Wuts, Protecting Groups in Organic Synthesis, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. Treatment of **14** with **13** following the procedures described in US Patent No. 6197779 and WO 0027825 give **15**. Removal of the protecting group gives phenol **16**. Reaction of phenol **16** with phosphonate reagent **9** in the presence of base in a protic solvent provides **4a**. Nitration (Scheme 4) of commercially available 2,6-dimethyl phenol provides **18**. Reduction of nitro group to amine, followed by protection of the resultant amine with protecting group, for example, such as trityl, Boc, Cbz etc as described in Greene and Wuts, Protecting Groups in Organic Synthesis, 3<sup>rd</sup> Edition, John Wiley and Sons Inc. Treatment of **14a** with **19** following the procedures described in US Patent No. 6197779 and WO 0027825 give **20**. Phenol **21** is obtained by treating **20** with

NH<sub>3</sub> using the procedure described in US Patent No. 6197779 and WO 0027825, followed by removal of the protecting group. Reaction of phenol **21** with phosphonate reagent **9** provides **4b**. As shown in Scheme 5, the commercially available 2,6-dimethyl-4-cyano-phenol **22** is reduced to benzyl amine, and the resultant amine is protected as described above. Phenol **23** is converted to phosphonate **4c** following the procedure described above for the transformation **19** to **4b**, just replace **19** with **23**. For example (Example 3), nitration of 2,6-dimethyl phenol with HNO<sub>3</sub> in H<sub>2</sub>SO<sub>4</sub> gives phenol **18**. The nitro group is reduced under catalytic hydrogenation condition, and subsequent protection of the resulting amine with Boc- gives phenol **19a**. Treatment of phenol **18** with sodium hydride, followed by reacting the resulting sodium phenoxide with **13** in dioxane provides **20a**. Removal of the Boc- with TFA followed by treatment of the resulting product with NH<sub>3</sub> in isopropyl alcohol according to US Patent No. 6197779 and WO 0027825 replaces the Cl- with NH<sub>2</sub> group to give **21**. The amine group in the phenyl ring is used as attachment site for introduction of phosphonate. Reductive amination of amine with aldehyde **9.3** provides **4b.1**. Treatment of **21** with p-nitro-phenyl carbonate, followed by aminoethyl phosphonate **9.4** affords urea linker **4b.2**.

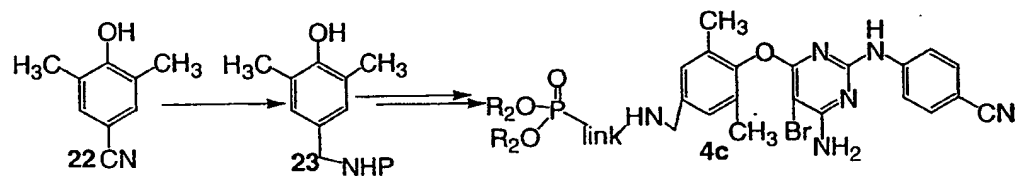
Scheme 3

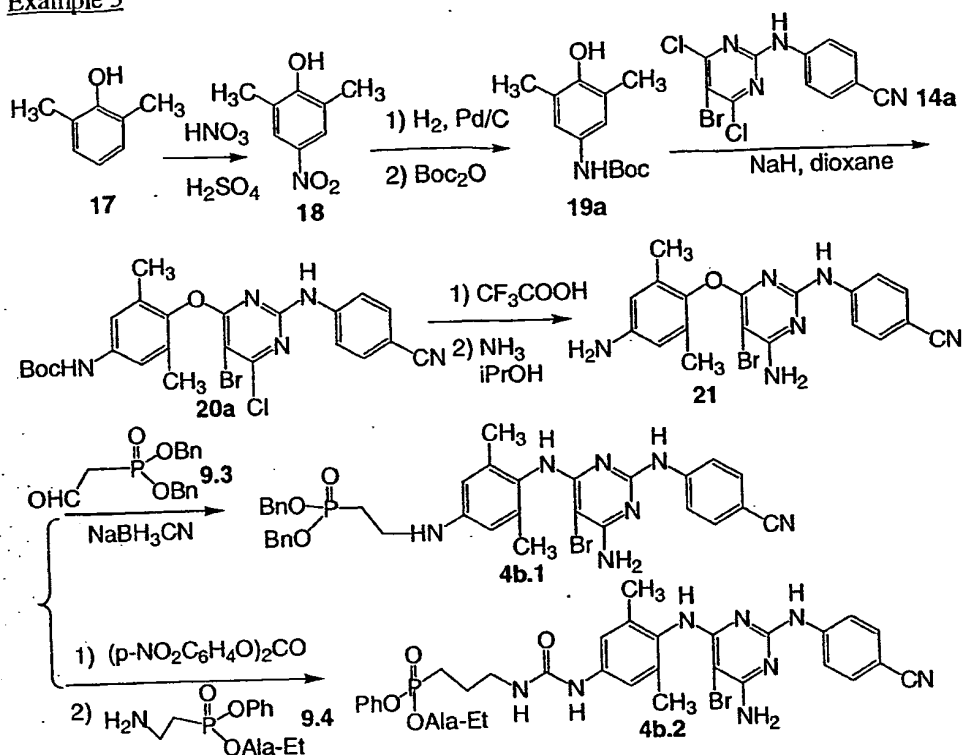


Scheme 4



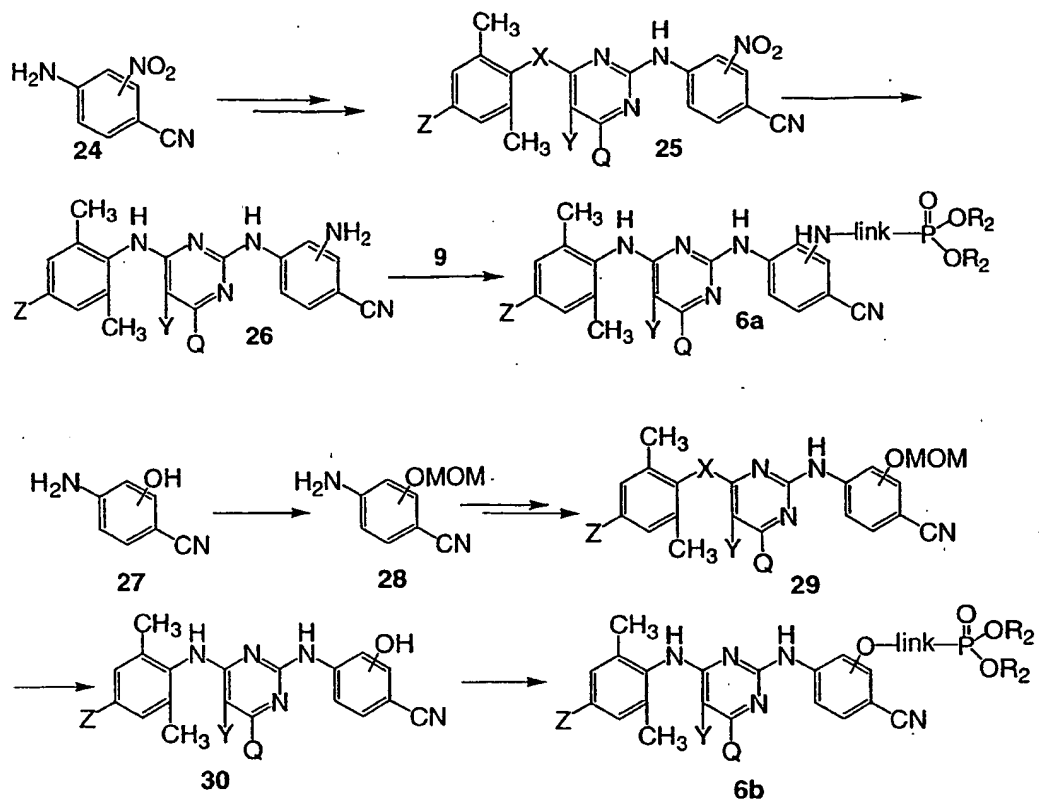
Scheme 5



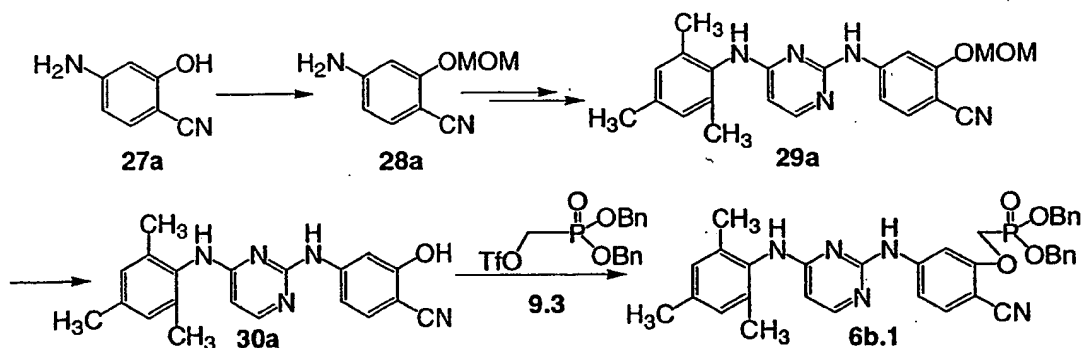
Example 3

Scheme 6 shows the preparation of phosphonate type 6 in Figure 2. Substituted 4-amino-benzonitriles **24** or **27**, which bearing a protected amino or hydroxyl group, or a precursor of amino group, are used in the replacement of 4-amino-benzonitrile for the preparation of TMC-125 and TMC-120 class of analogs as described in US Patent No. 6197779 and WO 0027825. TMC-120 and TMC-125 analogs **25** and **29** are thus obtained. Removal of protecting group or conversion to amine group from a precursor, such as a nitro group, provide **26** or **30** respectively. Treatment of **26** and/or **30** with reagent **9** yield **6a** and/or **6b** respectively. For example (Example 4), the hydroxyl group of 4-amino-2-hydroxy-benzonitrile **27a** is protected as its MOM-ether to give **28a**. Following the procedure in US Patent No. 6197779 and WO 0027825, **28a** is converted to TMC-120 analog **29a**. Removal of MOM-ether with TFA provides phenol **30a**, which is treated with trifluoromethylsulfonyl phosphonic acid benzyl ester together with  $\text{Cs}_2\text{CO}_3$  in acetonitrile affords phosphonate analog **6b.1**.

Scheme 6.



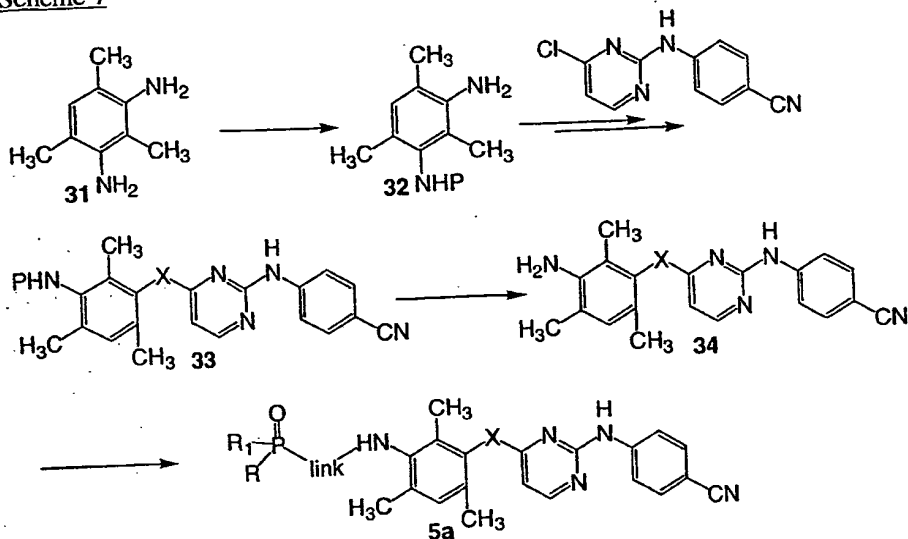
## Example 4



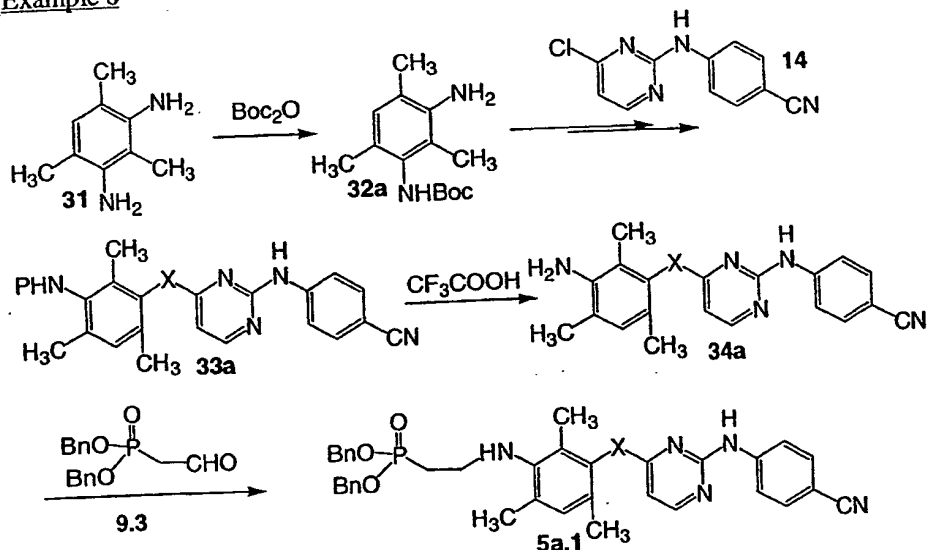
Preparation of phosphonate analog type 5 in Figure 2 is shown in Scheme 7. Substituted aniline, which bearing a protected amino or hydroxyl group, is converted to TMC-120 or TMC-125 analogs following the procedures described in US Patent No. 6197779 and WO 0027825. Removal of the protecting group gives analog 34. The amino or hydroxyl group in 33 serves as attachment site for introduction of phosphonate. Reaction of 33 with

reagent 9 provides 5a. For example (Example 5), commercially available 2-amino-2,4,6-trimethyl-aniline is selectively protected as Boc- carbamate. Reaction of 32a with 13 provides 33a. Removal of Boc with TFA affords aniline 34a. Reductive amination with reagent 9.2 yields phosphonate analog 5a.1.

### Scheme 7

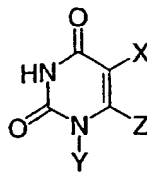
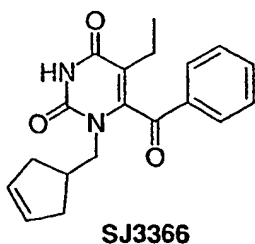


### Example 6



SJ3366-like phosphonate NNRTI compounds





X = alkyl C<sub>1</sub>-C<sub>12</sub> branched or straight

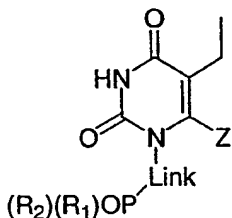
Y = alkyl, alkoxy, with or without link-PO(R<sub>1</sub>)(R<sub>2</sub>)

Z = Y<sub>2</sub>-link-PO(R<sub>1</sub>)(R<sub>2</sub>) or

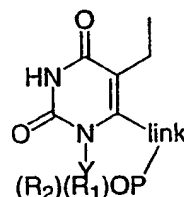
Y<sub>2</sub>-Aryl (optionally substituted)

or Y<sub>2</sub>-alkyl

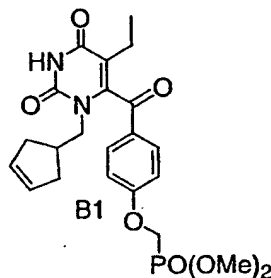
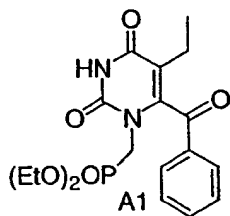
Y<sub>2</sub>=CR<sub>2</sub>, O, S, NR (R = H, alkyl C<sub>1</sub>-C<sub>12</sub>), C=O, COH



**Type A** [Y = link PO(R<sub>1</sub>)(R<sub>2</sub>)]



**Type B** [Z=link PO(R<sub>1</sub>)(R<sub>2</sub>)]



SJ3366 is described in US Patent No. 5922727. The present invention provides novel phosphonate analogs of SJ3366 which possess all the utilities of SJ3366 and optionally provide cellular accumulation as set forth below.

The present invention also relates to the delivery of SJ3366-like phosphonate compounds which are optionally targeted for site-specific accumulation in cells, tissues or organs. More particularly, this invention relates to analogs of SJ3366 which comprise SJ3366 linked to a PO(R<sub>1</sub>)(R<sub>2</sub>) moiety.

SJ3366 may be covalently bonded directly or indirectly by a link to the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety. An R group of the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety can possibly be cleaved within the desired delivery site, thereby forming an ionic species which does not exit the cell easily. This may cause accumulation within the cell and can optionally protect the SJ3366 analog from exposure to metabolic enzymes which would metabolize the analog if not protected within the

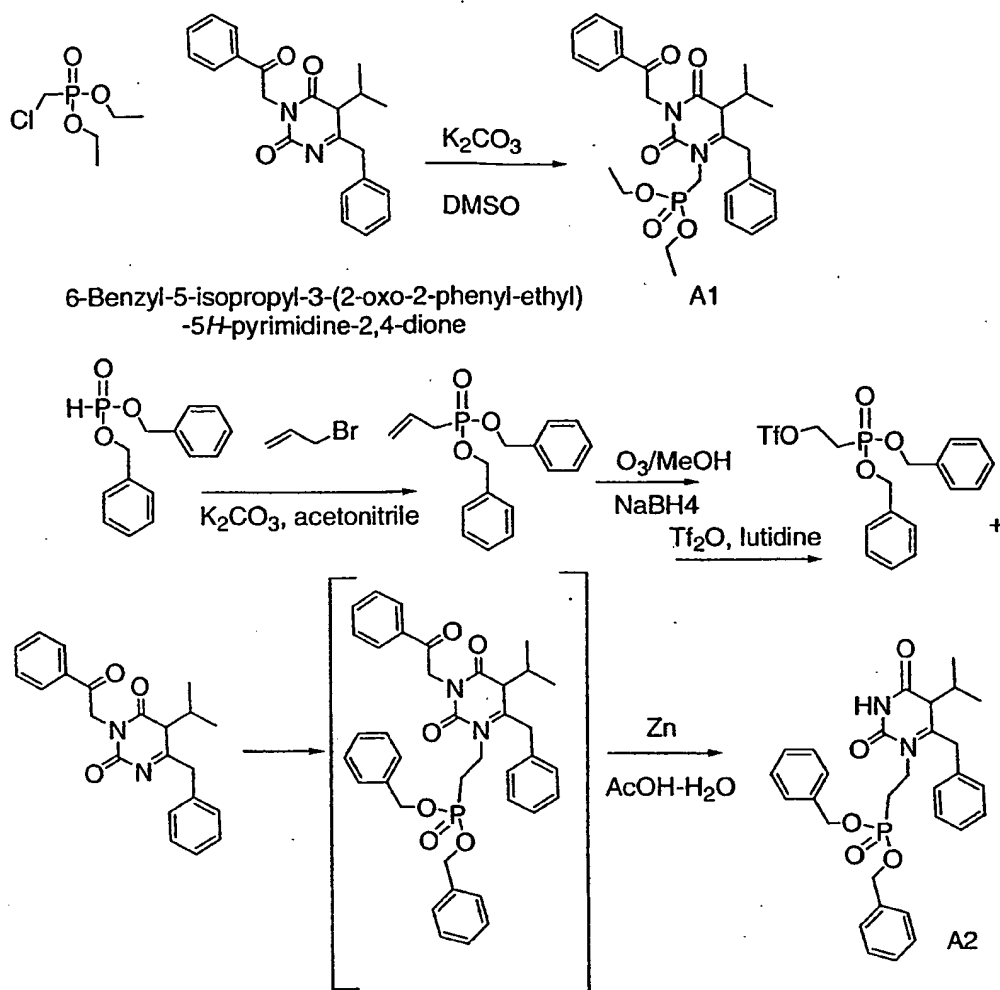
cell. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic action, but is preferably caused to occur selectively at a predetermined release site. The advantage of this method is that the SJ3366 analog may optionally be delivered site-specifically, may optionally accumulate within the cell and may optionally be shielded from metabolic enzymes.

The following examples illustrate various aspects of the present invention and are not to be construed to limit the types of analogs that may employ this strategy of linking SJ3366 or an SJ3366 analog to a  $PO(R_1)(R_2)$  moiety in any manner whatsoever.

Preparation of compounds of type A require a link which can react with SJ3366 or an intermediate or analog thereof, to result in a covalent bond between the link and the drug-like compound. The link is also attached to the phosphorous containing moiety as shown in an example of type A, namely A1.

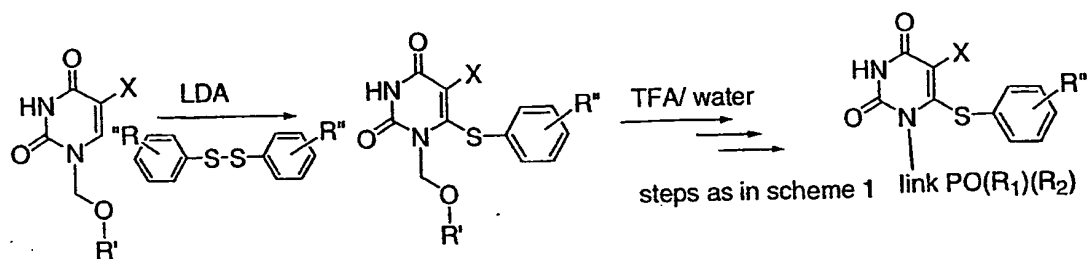
Examples of type A can be made by 1-alkylation of the 3-phenacyl derivatives 35 and 36 (synthesis described in J. Med. Chem. 1995, 38, 1860-2865, and so numbered 35 and 36 therein) with alkyl halide containing links followed by deprotection of the 3-phenacyl group. An example synthesis is as follows, and is shown in Scheme 1. 6-Benzyl-5-isopropyl-3-(2-phenyl-allyl)-dihydro-pyrimidine-2,4-dione, as prepared in J. Med. Chem. 1995, 38, 15, 2860-2865, is treated analogously to the reference article authors' treatment in preparing their compounds 37-40, but in the case of compound A1, commercially available chloromethyldiethylphosphonate is used as the alkylating agent. Alternatively the link is connected by starting with the same drug-like compound and using a triflated link. The triflated link is prepared, for example, by reaction of allyl bromide with dibenzylphosphite and potassium carbonate in acetonitrile at 65°C. Ozonolysis of the double bond followed by treatment with sodium borohydride would provide the alcohol, which could then be reacted with triflic anhydride with 2,6 lutidine in dichloromethane to produce the triflate. The triflated material could then be attached by stirring it with, for example 6-Benzyl-5-isopropyl-3-(2-phenyl-allyl)-dihydro-pyrimidine-2,4-dione with 2,6 lutidine or other base in an appropriate solvent such as acetone. This procedure will provide examples A1 and A2.

Scheme 1



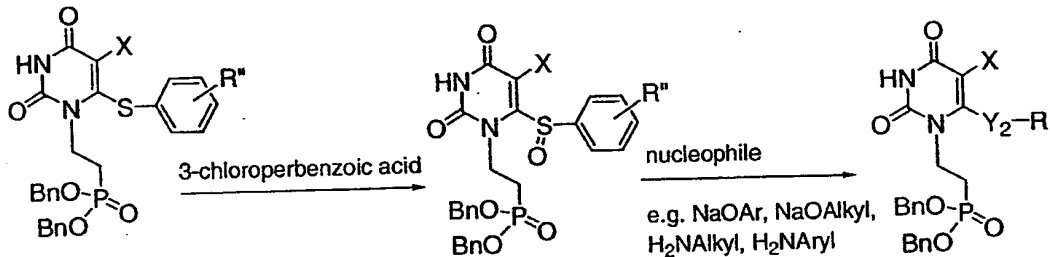
Scheme 1 can be extended to include analogs with various moieties at C6 in addition to substituted benzyl rings. For example, the LDA treatment described in J. Med. Chem. 1995, 38, 15, 2860-2865 followed by disulfide addition provides intermediates which can then be treated similarly to those in scheme 1 to install the link  $\text{PO(R}_1\text{)(R}_2\text{)}$  at the 1 position

Scheme 2



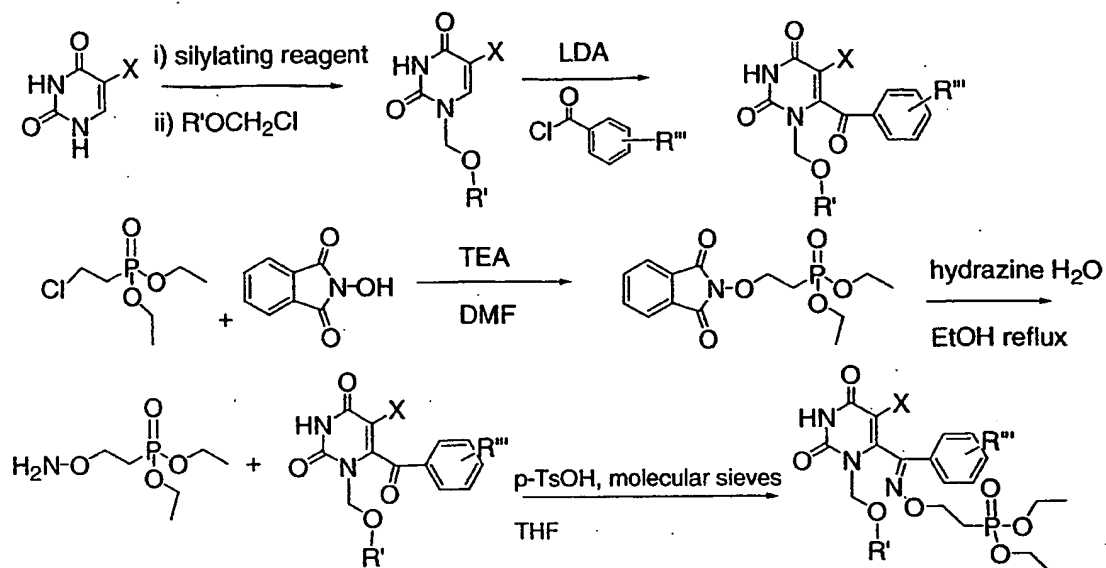
Scheme 3 also demonstrates a method to prepare analogs with oxygen or nitrogen at Y<sub>2</sub> attached to the 6 position. This method is explained fully in J. Med. Chem. 1991, 34,1, 349 - 357. Using this method allows for aryl and alkyl groups to be attached to the 6 position by either oxygen or nitrogen. A specific example is shown in the bottom row of the boxes in Scheme 7 below.

Scheme 3



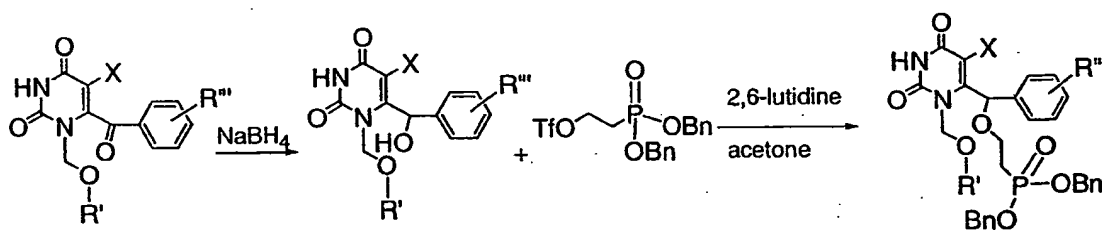
Alternatively the 5 position may be functionalized after the nucleophile is appended by the TFA/water deprotection and alkylation strategy shown in Scheme 2. Analogs with methylene, a secondary alcohol or a ketone at the 6 position are readily prepared following the LDA procedure in Scheme 2, but using substituted or unsubstituted PhCOCl in place of a disulfide, as is done in J. Med. Chem. 1991, 34, 1 page 351. The resultant ketone can be converted to an oxime ether (Scheme 4), an ether (Scheme 5) or reduced to a methylene (Scheme 6). Scheme 6 can be extended with the deprotection and alkylation steps described in Scheme 2. The methylene, secondary alcohol and ether are all described in J. Med. Chem. 1991, 34, 1 page 349-357, and the oxime ether can be prepared as described below (Scheme 4).

Scheme 4

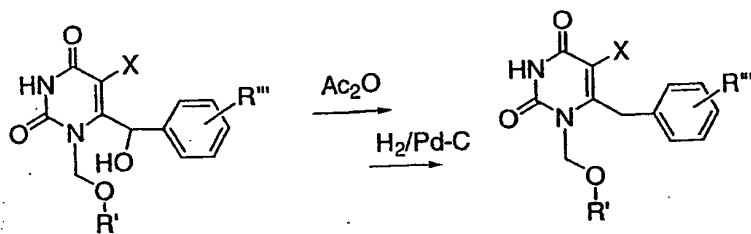


Alternatively the ketone containing compound could undergo deprotection at the 1 position and attachment of the link  $\text{PO}(\text{R}_1)(\text{R}_2)$  as in Scheme 2 above.

Scheme 5

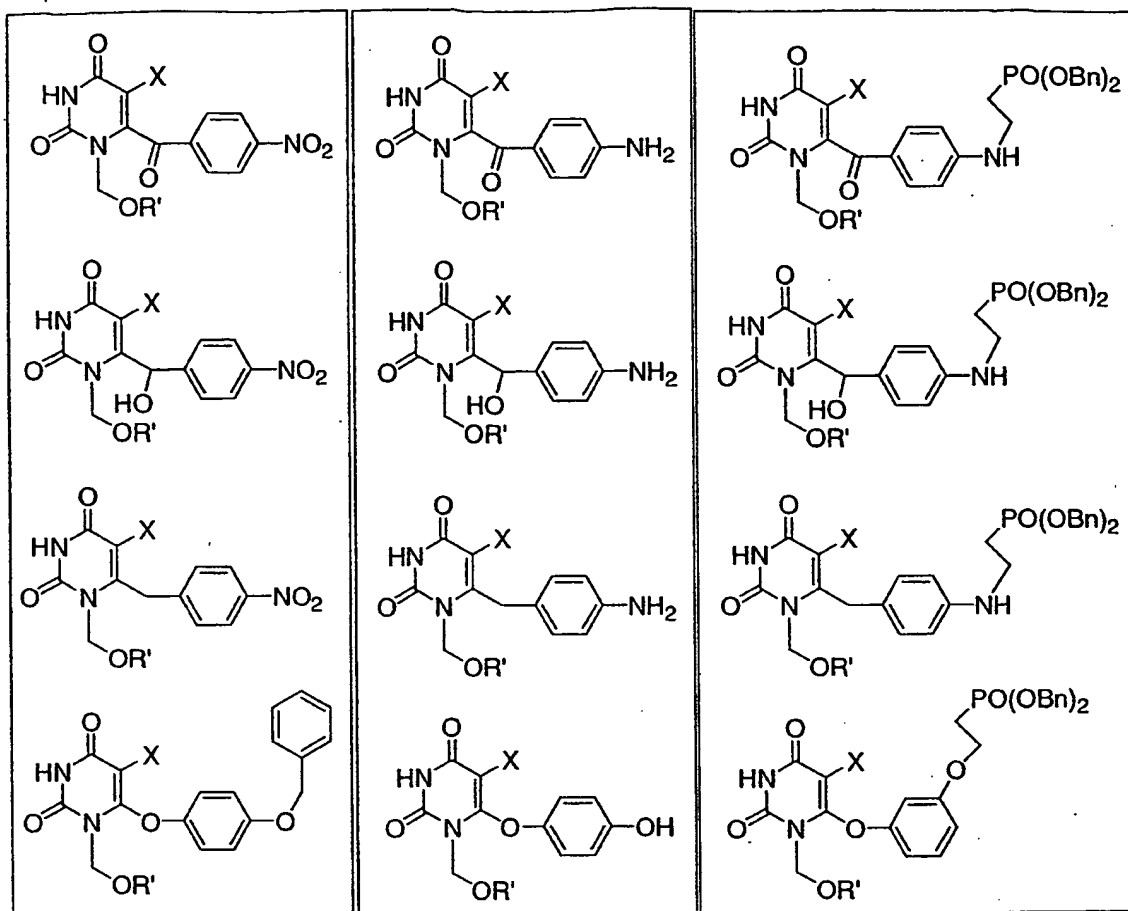


Scheme 6



The above shown compounds could also have a reactive group at the aryl or alkyl substituent on the 5 or the 6 position that would allow for attachment of the PO(R<sub>1</sub>)(R<sub>2</sub>) group. These reactive groups are protected by a protecting group, or be present in the form of a masked functionality, such as the manner in which a nitro group would mask an amine. Scheme 7 shows some more representative examples of the many ways an attachment of a PO(R<sub>1</sub>)(R<sub>2</sub>) is made. The chemistry involved is explained above, except for the BBr<sub>3</sub> demethylation, which is a common procedure (J.F.W. McOmie and D.E. West, *Org. Synth. Collect. Vol. V*, 412, (1973) for demethylating methoxyaryl rings. The compounds in box A are treated with hydrogen gas and stirred in a solvent such as ethanol or methanol with a suspension of 10% palladium on carbon. The anilines or alcohols are then treated with a triflated PO(R<sub>1</sub>)(R<sub>2</sub>) containing group as described above.

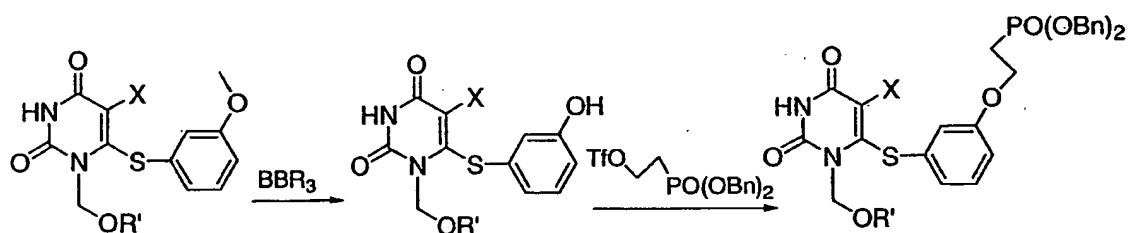
Scheme 7



A

B

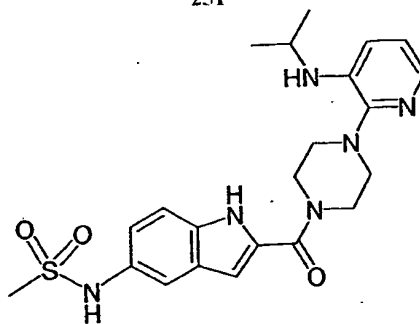
C



#### Delavirdine-like phosphonate NNRTI compounds

Diatomic compounds refer to any diatomic substituted compound, more specifically, bis(heteroaryl) piperazine (BHAP), more specifically 1{5-methanesulfonamidoindolyl-2-carbonyl}-4-{3-(1-methylethylamino)-2-pyridinyl}piperazine as found in US Patent No. 5563142 claim 8 column 90 line 49-51, and pharmaceutically acceptable salts thereof.

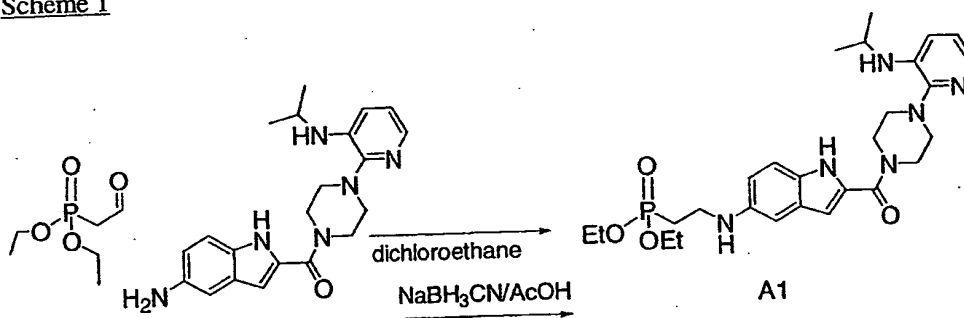
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Delavirdine

Preparation of compounds of type A, B, and C require a link which can react with a drug-like compound which is either 1{5-methanesulfonamidoindolyl-2-carbonyl}-4-{3-(1-methylethylamino)-2-pyridinyl}piperazine or an intermediate thereof, to result in a covalent bond between the link and the drug-like compound. The link is also attached to the phosphorous containing moiety shown in examples of type A, B and C, namely A1, B1 and C1.

Scheme 1



Examples of type A can be made by reacting the aminoindole  $\text{NH}_2$  of the immediate precursor to delavirdine (1-[5-amidoindolyl-2-carbonyl]-4-[3-(1-methylethylamino)-2-pyridinyl]piperazine, such as example 101 in US Patent No. 5563142, synthesis described therein, with the phosphorous containing moiety having an aldehyde as the reactive part of the link. The aldehyde and  $\text{NH}_2$  group react through a reductive amination reaction, which can be

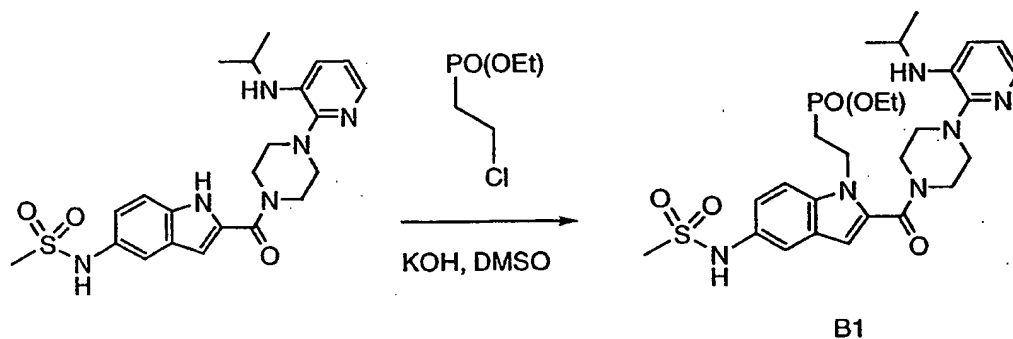


performed by stirring both reagents in, for example dichloroethane, for approximately two hours and then adding acetic acid and sodium cyanoborohydride, or by other standard methods known to most organic chemists. Commercially available aldehyde containing phosphonates such as that shown in the below scheme 1 can be used to prepare example A1.

This method may be extended to synthesize molecules with the link attached at other positions on the indole phenyl ring by following the procedures described in US Patent No. 5563142 but substituting starting materials as relevant to obtain the indole with the desired substitution pattern.

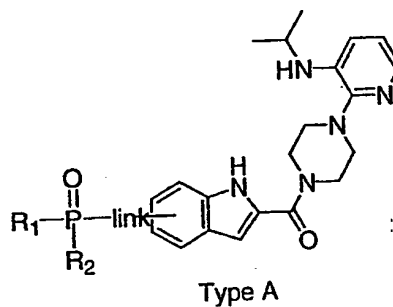
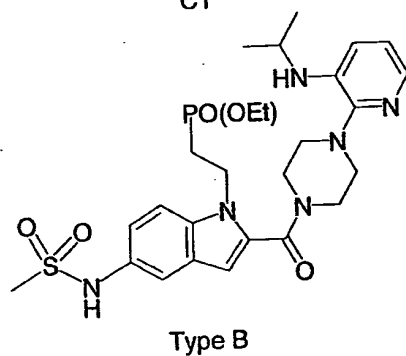
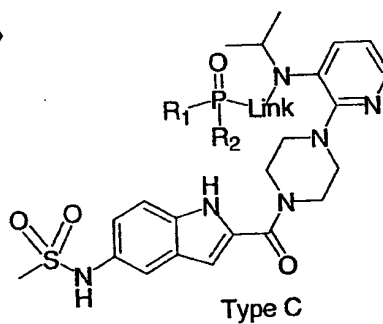
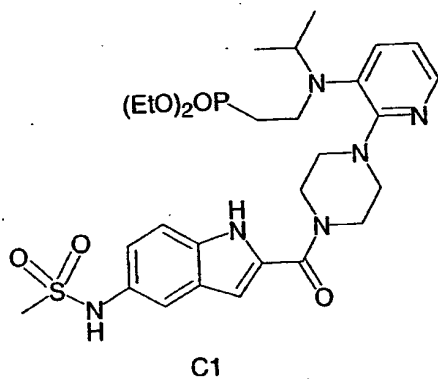
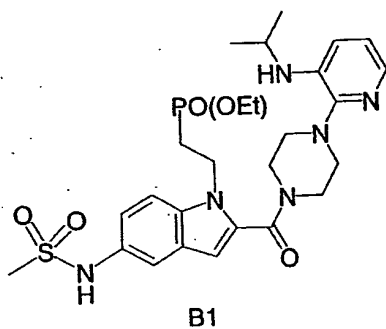
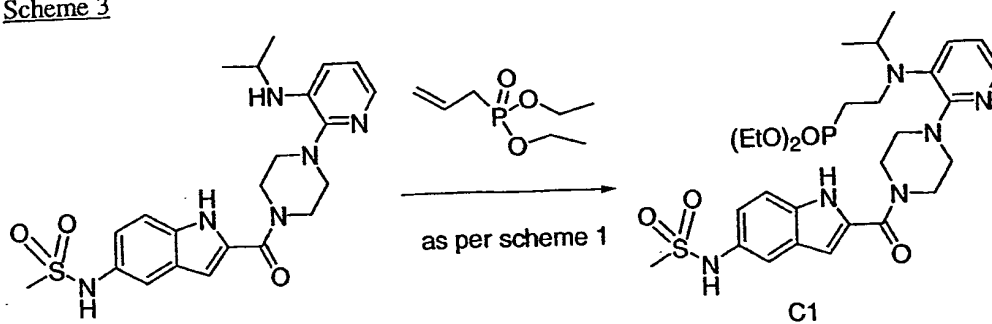
Examples of type B can be prepared by reacting the indole NH of delavirdine with, for example, a link which contains an alkyl chloride in the presence of KOH in DMSO as described in J. Med. Chem. 34, 3, 1991, 1099-1110. The alkyl chloride link is for example commercially available chloromethyl diethoxyphosphonate, giving example B1.

#### Scheme 2



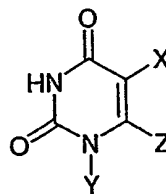
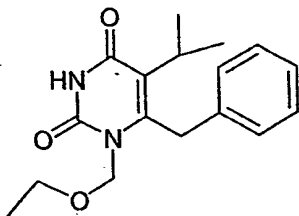
Examples of type C can be made by reacting the secondary amine of delavirdine with the phosphorous containing moiety having an aldehyde as the reactive part of the link. The aldehyde and NH group react through a reductive amination reaction, which can be performed by stirring both reagents in, for example dichloroethane, for approximately two hours and then adding acetic acid and sodium cyanoborohydride, or by other standard methods known to most organic chemists. In this example the aldehyde containing phosphonate is commercially available. This procedure will provide example C1.

Scheme 3

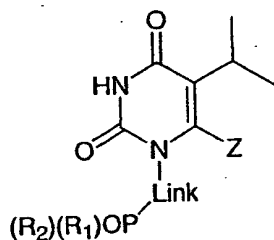
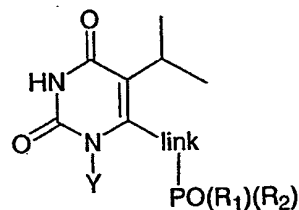
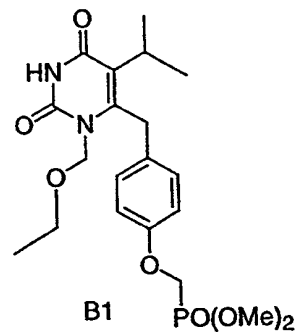
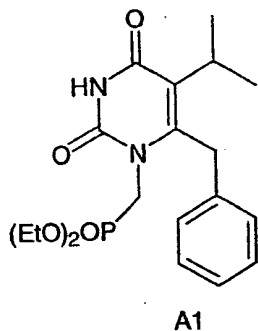


The present invention provides novel analogs of 1{5-methanesulfonamidoindolyl-2-carbonyl}-4-{3-(1-methylethylamino)-2-pyridinyl}piperazine. Such novel 1{5-methanesulfonamidoindolyl-2-carbonyl}-4-{3-(1-methylethylamino)-2-pyridinyl}piperazine

analogues possess all the utilities of 1 {5-methanesulfonamidoindolyl-2-carbonyl}-4- {3-(1-methylethylamino)-2-pyridinyl} piperazine and optionally provide cellular accumulation as set forth below.

Emivirine-like phosphonate NNRTI compounds

X = alkyl C<sub>1</sub>-C<sub>12</sub> branched or straight  
 Y = alkyl, alkoxy, with or without link-PO(R<sub>1</sub>)(R<sub>2</sub>) or  
 Z = Y<sup>2</sup>-link-PO(R<sub>1</sub>)(R<sub>2</sub>) or  
 Y<sup>2</sup>-Aryl (optionally substituted)  
 or Y<sup>2</sup>-alkyl  
 Y<sup>2</sup> = CR<sub>2</sub>, O, S, NR (R = H, alkyl C<sub>1</sub>-C<sub>12</sub>), C=C  
 COH

**Type A** [Y = link PO(R<sub>1</sub>)(R<sub>2</sub>)]**Type B** [Z = link PO(R<sub>1</sub>)(R<sub>2</sub>)]

The present invention provides novel phosphonate analogs of Emivirine and pharmaceutically acceptable salts thereof. Emivirine is described in US Patent No. 5461060. Such novel Emivirine analogs possess all the utilities of Emivirine and optionally provide cellular accumulation as set forth below.

The present invention also relates to the delivery of Emivirine-like phosphonate compounds which are optionally targeted for site-specific accumulation in cells, tissues or organs. More particularly, this invention relates to analogs of Emivirine which comprise Emivirine linked to a PO(R<sub>1</sub>)(R<sub>2</sub>) moiety.

Emivirine is covalently bonded directly or indirectly by a link to the  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety. An R group of the  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety can possibly be cleaved within the desired delivery site, thereby forming an ionic species which does not exit the cell easily. This may cause accumulation within the cell and can optionally protect the Emivirine analog from exposure to metabolic enzymes which would metabolize the analog if not protected within the cell. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic action, but is preferably caused to occur selectively at a predetermined release site. The advantage of this method is that the Emivirine analog may optionally be delivered site-specifically, may optionally accumulate within the cell and may optionally be shielded from metabolic enzymes.

Link: an atom or molecule which covalently binds together two components. In the present invention, a link is intended to include atoms and molecules which can be used to covalently bind Emivirine or an analog thereof at one end of the link to the  $\text{PO}(\text{R}_1)(\text{R}_2)$  at the other end of the link. The link must not prevent the binding of the analog with its appropriate receptor. Examples of suitable links include, but are not limited to, polymethylene  $[-(\text{CH}_2)_n]$ , where n is 1-10], ester, amine, carbonate, carbamate, ether, olefin, aromatic ring, acetal, heteroatom containing ring, or any combination of two or more of these units. The  $\text{PO}(\text{R}_1)(\text{R}_2)$  may also be directly attached. A skilled artisan will readily recognize other links which can be used in accordance with the present invention.

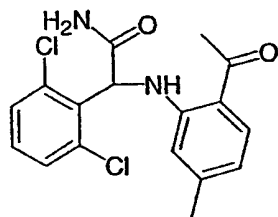
The preceding Schemes 1-7 for SJ3366-like phosphonate NNRTI compounds illustrate various aspects of the present invention and are not to be construed to limit the types of analogs that may employ this strategy of linking Emivirine or an Emivirine analog to a  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety in any manner whatsoever.

#### Loviride-like phosphonate NNRTI compounds

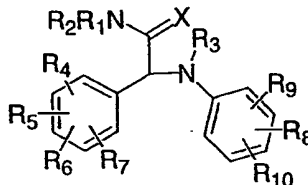
The present invention relates to Loviride-like phosphonate NNRTI compounds and their delivery to cells, tissue or organs which are optionally targeted for site-specific accumulation. More particularly, this invention relates to phosphonate analogs of Loviride, and their pharmaceutically acceptable salts and formulations, which comprise Loviride linked to a phosphonate, i.e.  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety.

The groups  $\text{R}_1$ - $\text{R}_{10}$  are as described in US Patent No. 5556886, and also can be link  $\text{PO}(\text{R}_1)(\text{R}_2)$ . The present invention provides novel phosphonate analogs of Loviride. Such

novel Loviride analogs possess all the utilities of NNRTI properties as Loviride and optionally provide cellular accumulation as set forth below.

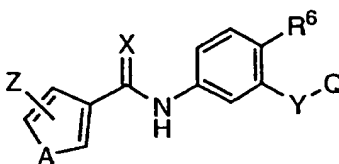


Loviride



Loviride may be covalently bonded directly or indirectly by a link to the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety. An R group of the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety can possibly be cleaved within the desired delivery site, thereby forming an ionic species which does not exit the cell easily. This may cause accumulation within the cell and can optionally protect the Loviride analog from exposure to metabolic enzymes which would metabolize the analog if not charged or protected within the cell. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic action, but is preferably caused to occur selectively at a predetermined release site. The advantage of this method is that the Loviride analog may optionally be delivered site-specifically, may optionally accumulate within the cell and may optionally be shielded from metabolic enzymes.

The following examples illustrate various aspects of the present invention and are not to be construed to limit the types of analogs that may employ this strategy of linking Loviride or an Loviride analog to a PO(R<sub>1</sub>)(R<sub>2</sub>) moiety in any manner whatsoever.

UC781-like phosphonate NNRTI compounds

The present invention includes UC781-like phosphonate compounds and pharmaceutically acceptable salts thereof. UC781 is described in US Patent No. 6143780.

A, X, Y, Q and R<sup>6</sup> in the formula above are as defined in US Patent No. 6143780. Z represents any substitution of the heteroatom ring. Also the heteroatom ring may be six membered. The present invention provides novel phosphonate analogs of UC781. Such novel UC781 analogs possess all the utilities of Emivirine and optionally provide cellular accumulation as set forth below. The present invention also relates to the delivery of UC781-like phosphonate compounds which are optionally targeted for site-specific accumulation in cells, tissues or organs. More particularly, this invention relates to analogs of UC781 which comprise UC781 linked to a PO(R<sub>1</sub>)(R<sub>2</sub>) moiety.

UC781 is covalently bonded directly or indirectly by a link to the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety. An R group of the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety can possibly be cleaved within the desired delivery site, thereby forming an ionic species which does not exit the cell easily. This may cause accumulation within the cell and can optionally protect the UC781e analog from exposure to metabolic enzymes which would metabolize the analog if not protected within the cell. The cleavage may occur as a result of normal displacement by cellular nucleophiles or enzymatic action, but is preferably caused to occur selectively at a predetermined release site. The advantage of this method is that the UC781 analog may optionally be delivered site-specifically, may optionally accumulate within the cell and may optionally be shielded from metabolic enzymes.

Link is any moiety which covalently binds together UC781 or an analog of UC781 and a phosphonate group. In the present invention, a link is intended to include atoms and molecules which can be used to covalently bind UC781 or an analog thereof at one end of the link to the PO(R<sub>1</sub>)(R<sub>2</sub>) at the other end of the link. The link should not prevent the binding of the analog with its appropriate receptor. Examples of suitable links include, but are not limited to, polymethylene [--(CH<sub>2</sub>)<sub>n</sub>, where n is 1-10], ester, amine, carbonate, carbamate, ether, olefin, aromatic ring, acetal, heteroatom containing ring or any combination of two or

more of these units. Direct attachment of the  $\text{PO}(\text{R}_1)(\text{R}_2)$  is also possible. A skilled artisan will readily recognize other links which can be used in accordance with the present invention.

The following examples illustrate various aspects of the present invention and are not to be construed to limit the types of analogs that may employ this strategy of linking UC781 or an UC781 analog to a  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety in any manner whatsoever.

Preparation of compounds of type A may proceed via a link which can react with UC781 or an analog or intermediate thereof, to result in a covalent bond between the link and the drug-like compound. The link is also attached to the phosphorous containing moiety as shown in an example of type A, namely A1.

Preparation of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarbothioamide, compound 12 in scheme 1 and intermediates 2, 4-11, as per US Patent No. 6143780.

Step 1: Preparation of 2-chloro-5-nitrobenzoyl alcohol 30 g of 2-chloro-5-nitrobenzaldehyde was dissolved in 500 mL of methanol and cooled to 0°C. A solution of 10 g of sodium borohydride in 100 mL of water was then added dropwise over 90 minutes while maintaining the temperature below 10°C. The resultant reaction mixture was then stirred for one hour, then acidified with 2N HCl and left stirring overnight. The solids were then, washed with water and dried, to produce 27 g of 2-chloro-5-nitrobenzoyl alcohol as a white solid.

Step 2: Preparation of 2-chloro-5-nitrobenzoyl acetate 27 g of the 2-chloro-5-nitrobenzoyl alcohol prepared above in Step 1, was dissolved in 122 mL of toluene. 22 mL of triethylamine was then added. The resultant reaction mixture was cooled to 20°C. and then a solution of 10.2 mL of acetyl chloride in 10 mL of toluene, was added dropwise, keeping the temperature below 20°C. The reaction mixture was then stirred overnight. 2.1 mL of triethylamine and 1.1 mL of acetyl chloride/toluene solution were then added and the reaction mixture was stirred for one hour. 100 mL of water was then added, followed by 50 mL of ether. The resulting organic phase was separated, washed with 2N HCl, aqueous sodium bicarbonate solution and water. The washed organic phase was then dried over magnesium sulfate and the solvent was evaporated, to produce 29.6 g of 2-chloro-5-nitrobenzoyl acetate as a white solid.

Step 3: Preparation of 5-amino-2-chlorobenzoyl acetate 24 g of iron powder was added to a solution of 1.6 mL of concentrated HCl, 16.8 mL of water, and 70 mL of ethanol. 29.6 g of the 2-chloro-5-nitrobenzoyl acetate prepared above in Step 2 dissolved in 45 mL of ethanol,



was then added to the mixture in three equal portions. The resultant reaction mixture was refluxed for 5 hours. An additional 2.4 g of iron and 0.1 mL of concentrated HCl was then added to the reaction mixture. The reaction mixture was then refluxed for an additional one hour, filtered through Celite and evaporated. 100 mL of water was then added to the evaporated material and the resultant mixture was extracted with 100 mL of ether. The ether solution was washed with water, dried over magnesium sulfate, and evaporated, to produce 22.9 g of 5-amino-2-chlorobenzoyl acetate as an oil.

Step 4: Preparation of N-(3-acetoxymethyl-4-chlorophenyl)-2-methyl-3-furancarboxanilide. A solution of 22.8 g of the 5-amino-2-chlorobenzoyl acetate from Step 3 above and 17.2 mL of triethylamine in 118 mL ether was prepared and then added dropwise to a second solution of 16.6 g 2-methyl-3-thiophenecarboxylic acid chloride in 118 mL ether at 0°C. to 10°C. and the resultant mixture was stirred at room temperature overnight. 100 mL of water and 100 mL of ethyl acetate were then added to the mixture, the organic phase separated, washed with 2N hydrochloric acid and water, dried over magnesium sulfate, and the solvents removed *in vacuo*, to produce 29.87 g of N-(3-acetoxymethyl-4-chlorophenyl)-2-methyl-3-furancarboxamide as a beige solid.

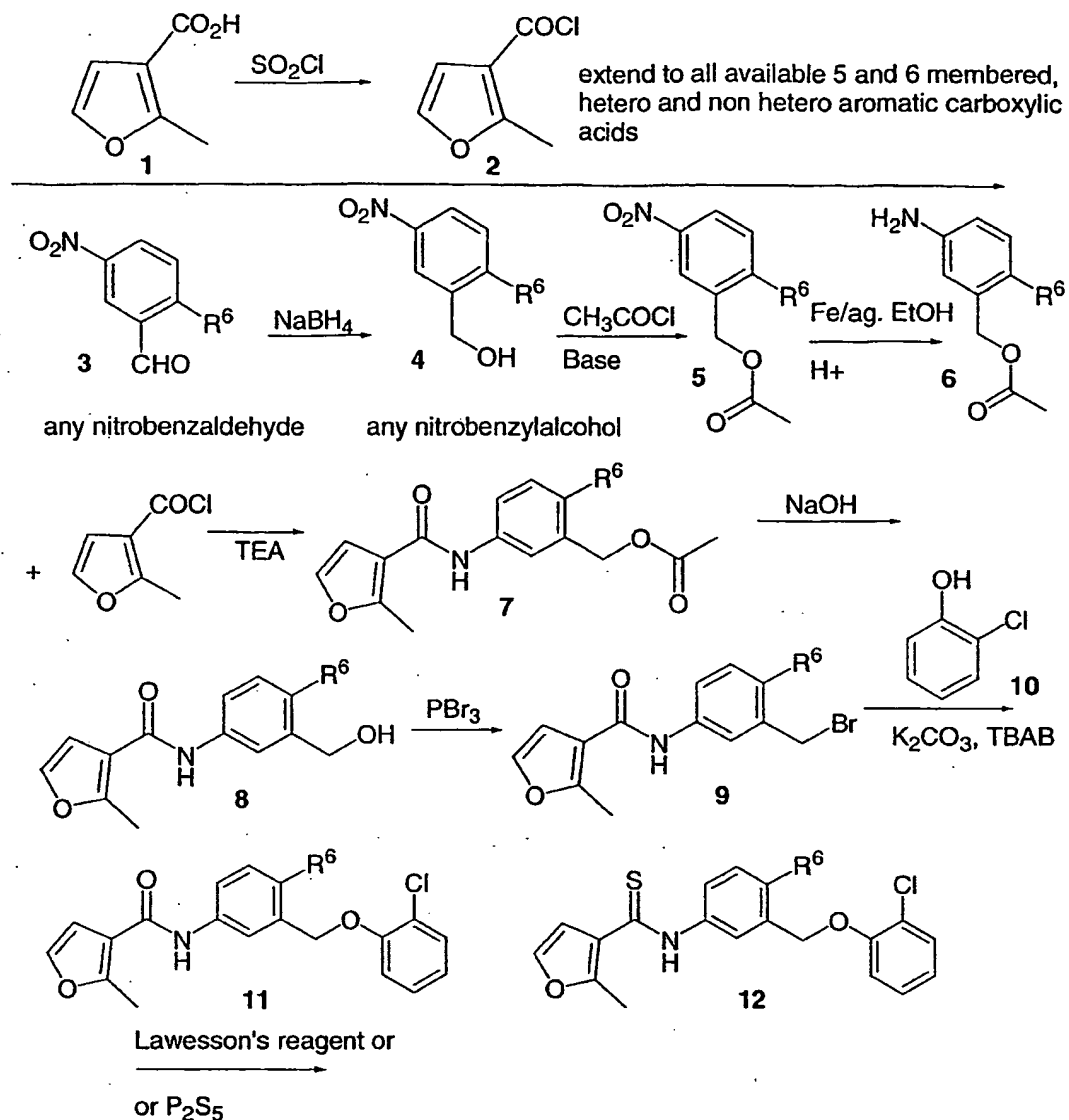
Step 5: Preparation of N-(4-chloro-3-hydroxymethylphenyl)-2-methyl-3-furancarboxamide. A solution of 29 g of the N-(3-acetoxymethyl-4-chlorophenyl)-2-methyl-3-furancarboxamide prepared in Step 4 above and 14.5 g potassium hydroxide in 110 mL water, was prepared. The solution was then heated at 70°C. for 16 hours and then acidified with 2N hydrochloric. The resulting solid was collected, washed with water, and dried, producing 23.65 g of N-(4-chloro-3-hydroxymethylphenyl)-2-methyl-3-furancarboxamide as a white solid.

Step 6: Preparation of N-(3-bromomethyl-4-chlorophenyl)-2-methyl-3-furancarboxamide. 12 g of the N-(4-chloro-3-hydroxymethylphenyl)-2-methyl-3-furancarboxamide prepared in Step 5 above, was dissolved in 180 mL ethyl acetate. 1.8 mL of phosphorus tribromide was then added. The resultant mixture was stirred for 90 minutes at room temperature. 100 mL of water was then added to the mixture. The resultant organic phase was separated, washed with water, aqueous sodium bicarbonate solution and water, and then dried over magnesium sulfate. The solvent was evaporated off to produce 12.97 g of N-(3-bromomethyl-4-chlorophenyl)-2-methyl-3-furancarboxamide as a solid.

Step 7: Preparation of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarboxamide. 2 g of the N-(3-bromomethyl-4-chlorophenyl)-2-methyl-3-furancarboxamide produced in Step 6, was dissolved in 20 mL of 2-butanone to produce a solution. 0.84 g of potassium carbonate, 0.79 g of 2-chlorophenol and 0.2 g of tetrabutylammonium bromide were then added to the solution. The resultant reaction mixture was stirred at room temperature overnight, the solvents removed *in vacuo*, and the residue extracted with ethyl acetate, to produce a second solution. This second solution was washed with 2N aqueous sodium hydroxide and water, and then dried over magnesium sulfate. The solvent was removed to produce 2.7 g of a solid, which was purified by dissolving in ethyl acetate:hexane (20:80) and running the resultant solution through a plug of silica gel. Removal of solvent produced 2.0 g of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarboxamide as a white solid.

Step 8: Preparation of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarbothioamide. 1.5 g of the N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarboxamide prepared in Step 7 above, 0.8 g of Lawesson's reagent (0.8 g) and 1.6 g of sodium bicarbonate were added to 35 mL of toluene, and the resultant reaction mixture was refluxed for five hours. The reaction mixture was then passed through a plug of neutral aluminum oxide, eluted with 1:1 ether/hexane and purified by column chromatography on silica gel, to produce 0.77 g of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarbothioamide.

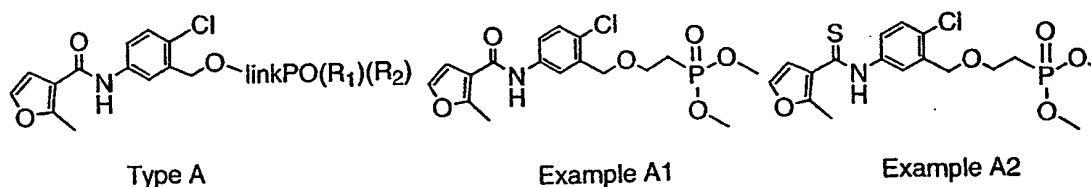
Scheme 1



The above protocol can easily be modified to attach the link-  $\text{PO}(\text{R}_1)(\text{R}_2)$ .

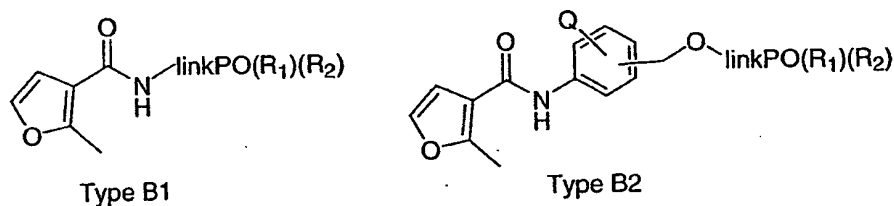
To prepare compounds of type A in Figure 1, the following route is performed. Compound 8 above, when  $\text{R}^6$  is chloro, is transformed into a triflate by reacting it with triflic anhydride and 2,6 lutidine in dichloromethane at  $-40^\circ\text{C}$ . The addition of hydroxyethyldimethoxyphosphonate will effect the attachment of the link  $\text{PO}(\text{R}_1)(\text{R}_2)$  group. Treatment with Lawesson's reagent as above will provide compound A2.

Figure 1



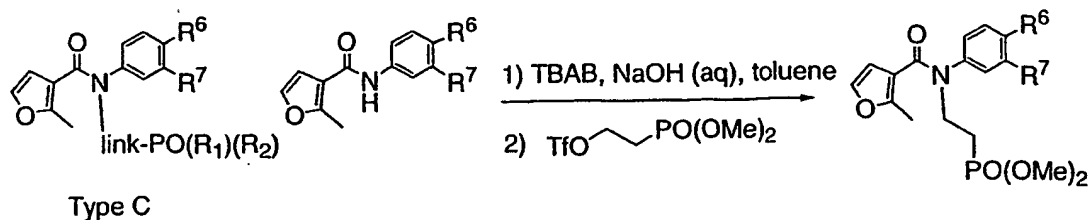
By replacing 2-chloro 5-nitrobenzaldehyde with other nitrobenzaldehydes and following a similar procedure as that used to make compound A2, the relative positions of attachment of the ether and the amide is changed. Furthermore, the chloro substituent shown as R<sup>6</sup> above is switched to other positions, and other substituents are used in combination with or without the chloro atom or other substituents anywhere on the ring (shown as Q below). This would allow for compounds of type B2 of Figure 2 to be prepared. As with all analogs that are amenable to such treatment, Lawesson's reagent would then be used to convert to the corresponding sulfamide.

Figure 2



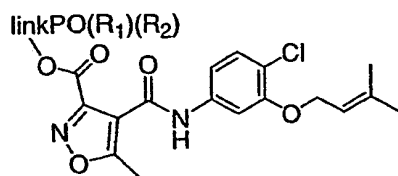
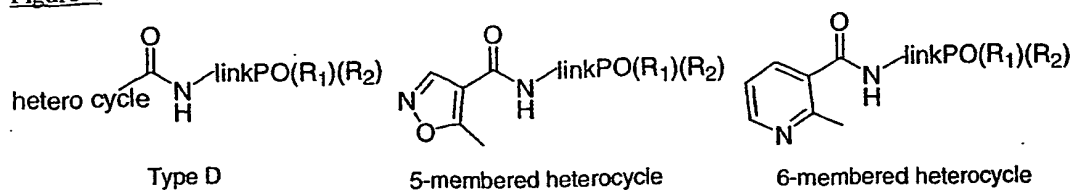
Type B1 compounds would include Type B2 and are prepared using the above steps with the center aryl ring being considered part of the link. Prior to treatment with Lawesson's reagent the amide proton is abstracted by treatment with base to allow for attachment of the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety. Lawesson's reagent would then be used to convert to the corresponding sulfamide. This would allow for compounds of the general form Type C shown in Figure 3.

Figure 3

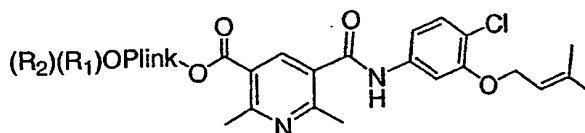


The furan ring of UC781 is switched to 5 or 6-membered heterocycles easily by substituting different heterocyclic acid chlorides for 2-methyl-3-thiophenecarboxylic acid chloride in step 4 in the above written synthesis of N-3-((2-chlorophenoxy)methyl)-4-chlorophenyl-2-methyl-3-furancarbothioamide. This will afford Type D compounds as exemplified below. The link  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety is attached directly to the heterocycle by starting with for example the diester of the desired heterocycle. Mono acid formation of the heterocycle by hydrolysis of one ester would allow for attachment of the  $\text{PO}(\text{R}_1)(\text{R}_2)$  group. This would be followed by hydrolysis of the remaining ester by base, acid chloride formation as above and amide formation by reaction with the desired amine. D1, a specific exemplification of Type D compounds having in this case  $\text{R}_1$  and  $\text{R}_2 = \text{OMe}$  and link =  $\text{CH}_2\text{CH}_2$  is prepared as shown below in Figure 4.

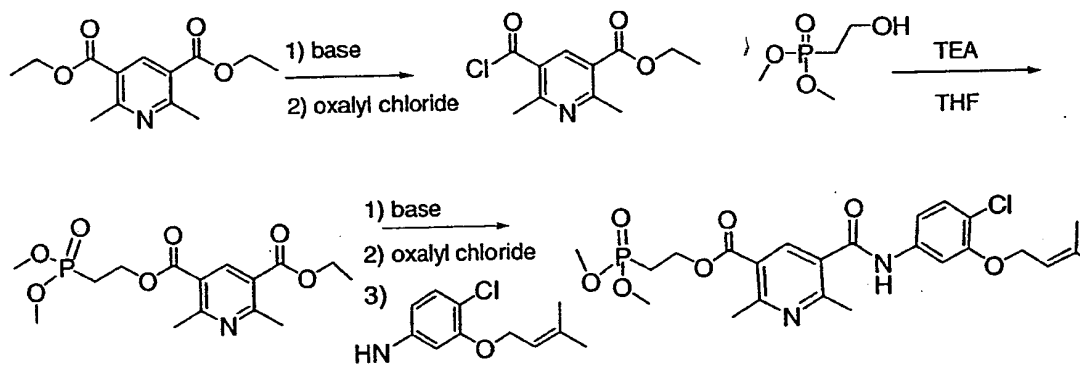
Figure 4



5-membered heterocycle  
with  $\text{linkPO(R}_1\text{)(R}_2\text{)}$  attached at  
heterocycle

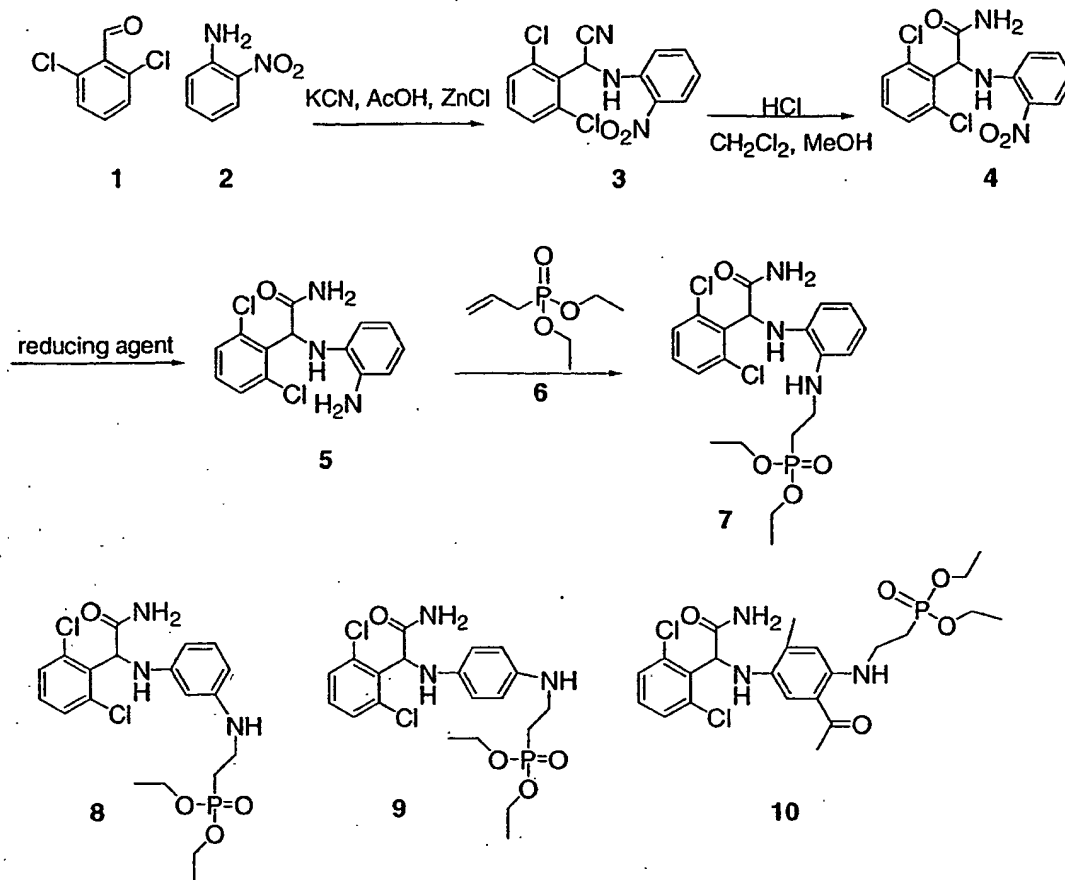


6-membered heterocycle  
with  $\text{linkPO(R}_1\text{)(R}_2\text{)}$  attached at  
heterocycle



All amides shown can be converted to sulfamides by treatment with Lawesson's reagent.

Scheme 1

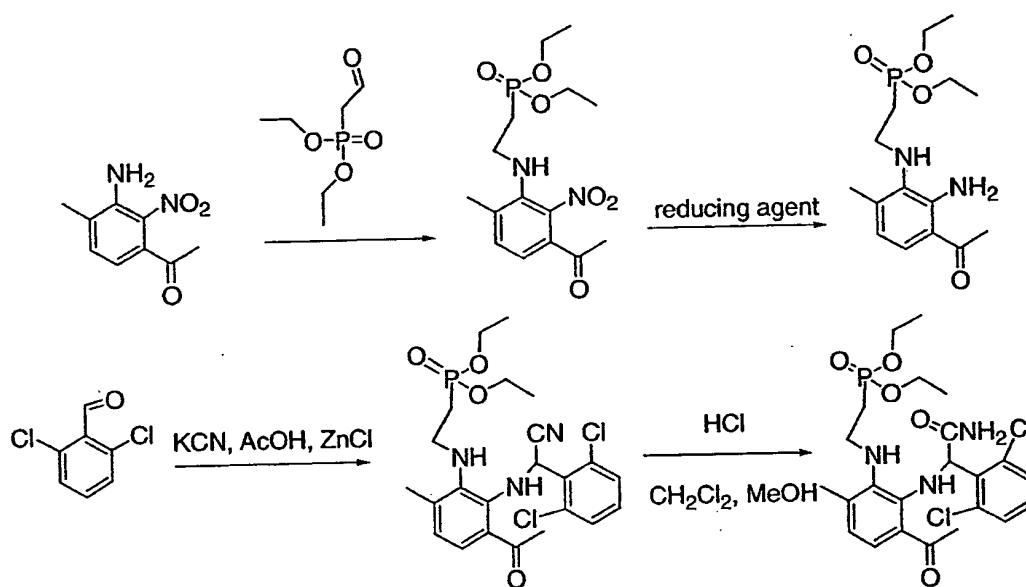


The details of the first two steps of Scheme 1 shown above are thoroughly covered in US Patent No. 5556886. The synthesis can be extended as shown to allow for the attachment of the link PO(R<sub>1</sub>)(R<sub>2</sub>) at various sites on either aryl ring.

To attach on the ortho, meta or para positions of the ring that starts out as the substituted aniline, a moiety must be present that will allow for such an attachment of the PO(R<sub>1</sub>)(R<sub>2</sub>) moiety. In this case a nitro group is used as an amine precursor. The reduction of the nitro can be effected by tin chloride and acetic acid in an appropriate solvent, or through some other catalytic hydrogenation method. From there, compounds such as compound 5 with a free anilino NH<sub>2</sub> can be reacted with, for example, a commercially available phosphonate such as compound 6 above in a reductive amination reaction. This reductive amination is performed using dichloroethane as solvent, and after stirring under dry conditions, sodium cyanoborohydride and acetic acid is added to complete the reaction giving compound 7. Using commercially available meta and para nitroanilines leads to compounds 8, 9 and 10.

Other substitution patterns are also possible. Also, other means of attachment are also possible to attach the drug-like compound to the  $\text{PO}(\text{R}_1)(\text{R}_2)$  piece. By varying the position of the nitro group, the  $\text{PO}(\text{R}_1)(\text{R}_2)$  is attached at any position on the anilino ring. Figure 1 below contains examples of nitroanilines that allow for the attachment at various positions.

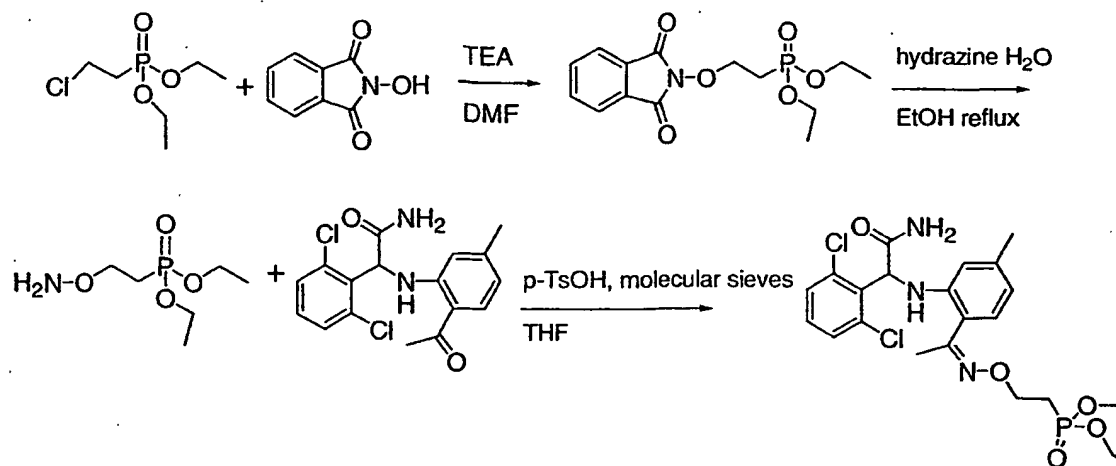
Alternatively, the nitroanilines is attached to the  $\text{PO}(\text{R}_1)(\text{R}_2)$  moiety prior to coupling with the aldehyde. The nitro is then reduced to form the aniline needed for coupling with the aldehyde. Hydrolysis of the cyano group to the amide is conducted as above, as illustrated in Scheme 2.



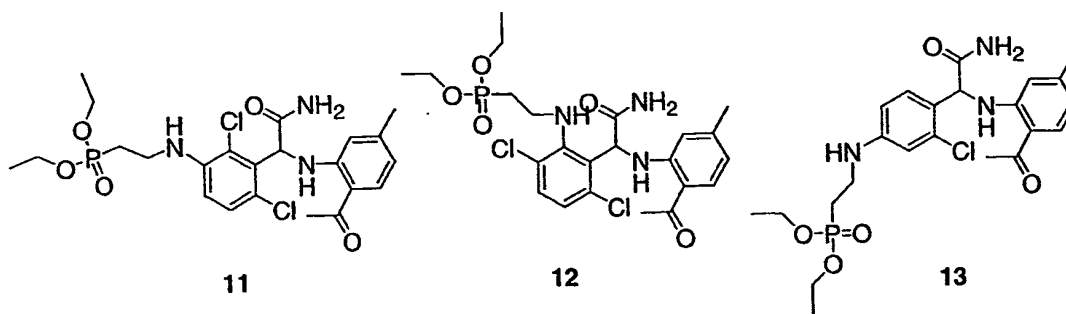
The ketone of Loviride or Loviride analogs also serves as a point of attachment for the  $\text{PO}(\text{R}_1)(\text{R}_2)$  group. The synthesis of such an attachment is shown in Scheme 3.



Scheme 3



By using a variation of the benzaldehyde shown as compound 1 in Scheme 1, further points of attachment are also attainable. By using, for example, 2,6-dichloro (3,4, or 5 nitro) benzaldehyde, and following Scheme 1, the PO(R<sub>1</sub>)(R<sub>2</sub>) is attached at any position of the ring which starts out as the benzaldehyde. Further examples of compounds that can be made in this way are compounds 11, 12 and 13 below.



### Scheme General Section

General aspects of these exemplary methods are described below and in the Example. Each of the products of the following processes is optionally separated, isolated, and/or purified prior to its use in subsequent processes.

The terms "treated", "treating", "treatment", and the like, mean contacting, mixing, reacting, allowing to react, bringing into contact, and other terms common in the art for indicating that one or more chemical entities is treated in such a manner as to convert it to one or more other chemical entities. This means that "treating compound one with compound two" is synonymous with "allowing compound one to react with compound two", "contacting compound one with compound two", "reacting compound one with compound two", and other expressions common in the art of organic synthesis for reasonably indicating that compound one was "treated", "reacted", "allowed to react", etc., with compound two.

"Treating" indicates the reasonable and usual manner in which organic chemicals are allowed to react. Normal concentrations (0.01M to 10M, typically 0.1M to 1M), temperatures (-100°C to 250°C, typically -78°C to 150°C, more typically -78°C to 100°C, still more typically 0°C to 100°C), reaction vessels (typically glass, plastic, metal), solvents, pressures, atmospheres (typically air for oxygen and water insensitive reactions or nitrogen or argon for oxygen or water sensitive), etc., are intended unless otherwise indicated. The knowledge of similar reactions known in the art of organic synthesis is used in selecting the conditions and apparatus for "treating" in a given process. In particular, one of ordinary skill in the art of organic synthesis selects conditions and apparatus reasonably expected to successfully carry out the chemical reactions of the described processes based on the knowledge in the art.

Modifications of each of the exemplary schemes above and in the examples (hereafter "exemplary schemes") leads to various analogs of the specific exemplary materials produce. The above cited citations describing suitable methods of organic synthesis are applicable to such modifications.

In each of the exemplary schemes it may be advantageous to separate reaction products from one another and/or from starting materials. The desired products of each step or series of steps is separated and/or purified (hereinafter separated) to the desired degree of homogeneity by the techniques common in the art. Typically such separations involve multiphase extraction, crystallization from a solvent or solvent mixture, distillation, sublimation, or chromatography. Chromatography can involve any number of methods including, for example, size exclusion or ion exchange chromatography, high, medium, or low pressure liquid chromatography, small scale and preparative thin or thick layer chromatography, as well as techniques of small scale thin layer and flash chromatography.

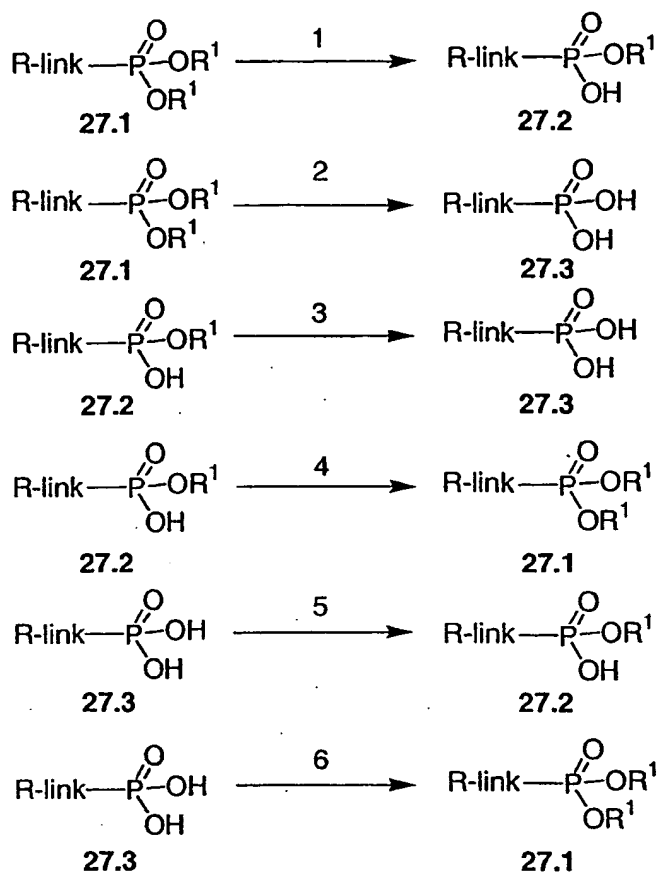
Another class of separation methods involves treatment of a mixture with a reagent

selected to bind to or render otherwise separable a desired product, unreacted starting material, reaction by product, or the like. Such reagents include adsorbents or absorbents such as activated carbon, molecular sieves, ion exchange media, or the like. Alternatively, the reagents can be acids in the case of a basic material, bases in the case of an acidic material, binding reagents such as antibodies, binding proteins, selective chelators such as crown ethers, liquid/liquid ion extraction reagents (LIX), or the like.

Selection of appropriate methods of separation depends on the nature of the materials involved. For example, boiling point, and molecular weight in distillation and sublimation, presence or absence of polar functional groups in chromatography, stability of materials in acidic and basic media in multiphase extraction, and the like. One skilled in the art will apply techniques most likely to achieve the desired separation.

All literature and patent citations above are hereby expressly incorporated by reference at the locations of their citation. Specifically cited sections or pages of the above cited works are incorporated by reference with specificity. The invention has been described in detail sufficient to allow one of ordinary skill in the art to make and use the subject matter of the following Embodiments. It is apparent that certain modifications of the methods and compositions of the following Embodiments can be made within the scope and spirit of the invention.

Scheme 1001



Scheme 1001 shows the interconversions of certain phosphonate compounds: acids -  $\text{P}(\text{O})(\text{OH})_2$ ; mono-esters -  $\text{P}(\text{O})(\text{OR}_1)(\text{OH})$ ; and diesters -  $\text{P}(\text{O})(\text{OR}_1)_2$  in which the  $\text{R}^1$  groups are independently selected, and defined herein before, and the phosphorus is attached through a carbon moiety (link, i.e. linker), which is attached to the rest of the molecule, e.g. drug or drug intermediate (R). The  $\text{R}^1$  groups attached to the phosphonate esters in Scheme 1001 may be changed using established chemical transformations. The interconversions may be carried out in the precursor compounds or the final products using the methods described below. The methods employed for a given phosphonate transformation depend on the nature of the substituent  $\text{R}^1$ . The preparation and hydrolysis of phosphonate esters is described in Organic Phosphorus Compounds, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 9ff.

The conversion of a phosphonate diester **27.1** into the corresponding phosphonate monoester **27.2** (Scheme 1001, Reaction 1) can be accomplished by a number of methods.

For example, the ester **27.1** in which  $R^1$  is an arylalkyl group such as benzyl, can be converted into the monoester compound **27.2** by reaction with a tertiary organic base such as diazabicyclooctane (DABCO) or quinuclidine, as described in *J. Org. Chem.*, 1995, 60:2946. The reaction is performed in an inert hydrocarbon solvent such as toluene or xylene, at about 110°C. The conversion of the diester **27.1** in which  $R^1$  is an aryl group such as phenyl, or an alkenyl group such as allyl, into the monoester **27.2** can be effected by treatment of the ester **27.1** with a base such as aqueous sodium hydroxide in acetonitrile or lithium hydroxide in aqueous tetrahydrofuran. Phosphonate diesters **27.2** in which one of the groups  $R^1$  is arylalkyl, such as benzyl, and the other is alkyl, can be converted into the monoesters **27.2** in which  $R^1$  is alkyl, by hydrogenation, for example using a palladium on carbon catalyst. Phosphonate diesters in which both of the groups  $R^1$  are alkenyl, such as allyl, can be converted into the monoester **27.2** in which  $R^1$  is alkenyl, by treatment with chlorotris(triphenylphosphine)rhodium (Wilkinson's catalyst) in aqueous ethanol at reflux, optionally in the presence of diazabicyclooctane, for example by using the procedure described in *J. Org. Chem.*, 38:3224 1973 for the cleavage of allyl carboxylates.

The conversion of a phosphonate diester **27.1** or a phosphonate monoester **27.2** into the corresponding phosphonic acid **27.3** (Scheme 1001, Reactions 2 and 3) can be effected by reaction of the diester or the monoester with trimethylsilyl bromide, as described in *J. Chem. Soc., Chem. Comm.*, 739, 1979. The reaction is conducted in an inert solvent such as, for example, dichloromethane, optionally in the presence of a silylating agent such as bis(trimethylsilyl)trifluoroacetamide, at ambient temperature. A phosphonate monoester **27.2** in which  $R^1$  is arylalkyl such as benzyl, can be converted into the corresponding phosphonic acid **27.3** by hydrogenation over a palladium catalyst, or by treatment with hydrogen chloride in an ethereal solvent such as dioxane. A phosphonate monoester **27.2** in which  $R^1$  is alkenyl such as, for example, allyl, can be converted into the phosphonic acid **27.3** by reaction with Wilkinson's catalyst in an aqueous organic solvent, for example in 15% aqueous acetonitrile, or in aqueous ethanol, for example using the procedure described in *Helv. Chim. Acta.*, 68:618, 1985. Palladium catalyzed hydrogenolysis of phosphonate esters **27.1** in which  $R^1$  is benzyl is described in *J. Org. Chem.*, 24:434, 1959. Platinum-catalyzed hydrogenolysis of phosphonate esters **27.1** in which  $R^1$  is phenyl is described in *J. Amer. Chem. Soc.*, 78:2336, 1956.

The conversion of a phosphonate monoester **27.2** into a phosphonate diester **27.1** (Scheme 1001, Reaction 4) in which the newly introduced  $R^1$  group is alkyl, arylalkyl, or

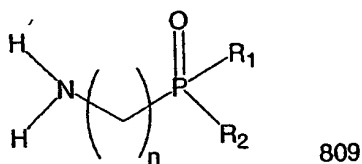
haloalkyl such as chloroethyl, can be effected by a number of reactions in which the substrate 27.2 is reacted with a hydroxy compound  $R^1OH$ , in the presence of a coupling agent. Suitable coupling agents are those employed for the preparation of carboxylate esters, and include a carbodiimide such as dicyclohexylcarbodiimide, in which case the reaction is preferably conducted in a basic organic solvent such as pyridine, or (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PYBOP, Sigma), in which case the reaction is performed in a polar solvent such as dimethylformamide, in the presence of a tertiary organic base such as diisopropylethylamine, or Aldrithiol-2 (Aldrich) in which case the reaction is conducted in a basic solvent such as pyridine, in the presence of a triaryl phosphine such as triphenylphosphine. Alternatively, the conversion of the phosphonate monoester 27.1 to the diester 27.1 can be effected by the use of the Mitsunobu reaction. The substrate is reacted with the hydroxy compound  $R^1OH$ , in the presence of diethyl azodicarboxylate and a triarylphosphine such as triphenyl phosphine. Alternatively, the phosphonate monoester 27.2 can be transformed into the phosphonate diester 27.1, in which the introduced  $R^1$  group is alkenyl or arylalkyl, by reaction of the monoester with the halide  $R^1Br$ , in which  $R^1$  is as alkenyl or arylalkyl. The alkylation reaction is conducted in a polar organic solvent such as dimethylformamide or acetonitrile, in the presence of a base such as cesium carbonate. Alternatively, the phosphonate monoester can be transformed into the phosphonate diester in a two step procedure. In the first step, the phosphonate monoester 27.2 is transformed into the chloro analog  $-P(O)(OR^1)Cl$  by reaction with thionyl chloride or oxalyl chloride and the like, as described in Organic Phosphorus Compounds, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 17, and the thus-obtained product  $-P(O)(OR^1)Cl$  is then reacted with the hydroxy compound  $R^1OH$ , in the presence of a base such as triethylamine, to afford the phosphonate diester 27.1.

A phosphonic acid  $-P(O)(OH)_2$  can be transformed into a phosphonate monoester  $-P(O)(OR^1)(OH)$  (Scheme 1001, Reaction 5) by means of the methods described above of for the preparation of the phosphonate diester  $-P(O)(OR^1)_2$  27.1, except that only one molar proportion of the component  $R^1OH$  or  $R^1Br$  is employed.

A phosphonic acid  $-P(O)(OH)_2$  27.3 can be transformed into a phosphonate diester  $-P(O)(OR^1)_2$  27.1 (Scheme 1, Reaction 6) by a coupling reaction with the hydroxy compound  $R^1OH$ , in the presence of a coupling agent such as Aldrithiol-2 (Aldrich) and triphenylphosphine. The reaction is conducted in a basic solvent such as pyridine. Alternatively, phosphonic acids 27.3 can be transformed into phosphonic esters 27.1 in which

$R^1$  is aryl, such as phenyl, by means of a coupling reaction employing, for example, phenol and dicyclohexylcarbodiimide in pyridine at about 70°C. Alternatively, phosphonic acids 27.3 can be transformed into phosphonic esters 27.1 in which  $R^1$  is alkenyl, by means of an alkylation reaction. The phosphonic acid is reacted with the alkenyl bromide  $R^1Br$  in a polar organic solvent such as acetonitrile solution at reflux temperature, in the presence of a base such as cesium carbonate, to afford the phosphonic ester 27.1.

Amino alkyl phosphonate compounds 809:



are a generic representative of compounds 811, 813, 814, 816 and 818. Some methods to prepare embodiments of 809 are shown in Scheme 1002. Commercial amino phosphonic acid 810 was protected as carbamate 811. The phosphonic acid 811 was converted to phosphonate 812 upon treatment with ROH in the presence of DCC or other conventional coupling reagents. Coupling of phosphonic acid 811 with esters of amino acid 820 provided bisamidate 817. Conversion of acid 811 to bisphenyl phosphonate followed by hydrolysis gave mono-phosphonic acid 814 (Cbz =  $C_6H_5CH_2C(O)-$ ), which was then transformed to mono-phosphonic amidate 815. Carbamates 813, 816 and 818 were converted to their corresponding amines upon hydrogenation. Compounds 811, 813, 814, 816 and 818 are useful intermediates to form the phosphonate compounds of the invention.

**Preparation of carboalkoxy-substituted phosphonate bisamidates, monoamidates, diesters and monoesters.**

A number of methods are available for the conversion of phosphonic acids into amidates and esters. In one group of methods, the phosphonic acid is either converted into an isolated activated intermediate such as a phosphoryl chloride, or the phosphonic acid is activated in situ for reaction with an amine or a hydroxy compound.



The conversion of phosphonic acids into phosphoryl chlorides is accomplished by reaction with thionyl chloride, for example as described in J. Gen. Chem. USSR, 1983, 53, 480, Zh. Obschei Khim., 1958, 28, 1063, or J. Org. Chem., 1994, 59, 6144, or by reaction with oxalyl chloride, as described in J. Am. Chem. Soc., 1994, 116, 3251, or J. Org. Chem., 1994, 59, 6144, or by reaction with phosphorus pentachloride, as described in J. Org. Chem., 2001, 66, 329, or in J. Med. Chem., 1995, 38, 1372. The resultant phosphoryl chlorides are then reacted with amines or hydroxy compounds in the presence of a base to afford the amidate or ester products.

Phosphonic acids are converted into activated imidazolyl derivatives by reaction with carbonyl diimidazole, as described in J. Chem. Soc., Chem. Comm., 1991, 312, or Nucleosides Nucleotides 2000, 19, 1885. Activated sulfonyloxy derivatives are obtained by the reaction of phosphonic acids with trichloromethylsulfonyl chloride, as described in J. Med. Chem. 1995, 38, 4958, or with triisopropylbenzenesulfonyl chloride, as described in Tet. Lett., 1996, 7857, or Bioorg. Med. Chem. Lett., 1998, 8, 663. The activated sulfonyloxy derivatives are then reacted with amines or hydroxy compounds to afford amidates or esters. Alternatively, the phosphonic acid and the amine or hydroxy reactant are combined in the presence of a diimide coupling agent. The preparation of phosphonic amidates and esters by means of coupling reactions in the presence of dicyclohexyl carbodiimide is described, for example, in J. Chem. Soc., Chem. Comm., 1991, 312, or J. Med. Chem., 1980, 23, 1299 or Coll. Czech. Chem. Comm., 1987, 52, 2792. The use of ethyl dimethylaminopropyl carbodiimide for activation and coupling of phosphonic acids is described in Tet. Lett., 2001, 42, 8841, or Nucleosides Nucleotides, 2000, 19, 1885.

A number of additional coupling reagents have been described for the preparation of amidates and esters from phosphonic acids. The agents include Aldrithiol-2, and PYBOP and BOP, as described in J. Org. Chem., 1995, 60, 5214, and J. Med. Chem., 1997, 40, 3842, mesitylene-2-sulfonyl-3-nitro-1,2,4-triazole (MSNT), as described in J. Med. Chem., 1996, 39, 4958, diphenylphosphoryl azide, as described in J. Org. Chem., 1984, 49, 1158, 1-(2,4,6-triisopropylbenzenesulfonyl-3-nitro-1,2,4-triazole (TPSNT) as described in Bioorg. Med. Chem. Lett., 1998, 8, 1013, bromotris(dimethylamino)phosphonium hexafluorophosphate (BroP), as described in Tet. Lett., 1996, 37, 3997, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-

dioxaphosphinane, as described in Nucleosides Nucleotides 1995, 14, 871, and diphenyl chlorophosphate, as described in J. Med. Chem., 1988, 31, 1305.

Phosphonic acids are converted into amidates and esters by means of the Mitsunobu reaction, in which the phosphonic acid and the amine or hydroxy reactant are combined in the presence of a triaryl phosphine and a dialkyl azodicarboxylate. The procedure is described in Org. Lett., 2001, 3, 643, or J. Med. Chem., 1997, 40, 3842.

Phosphonic esters are also obtained by the reaction between phosphonic acids and halo compounds, in the presence of a suitable base. The method is described, for example, in Anal. Chem., 1987, 59, 1056, or J. Chem. Soc. Perkin Trans., I, 1993, 19, 2303, or J. Med. Chem., 1995, 38, 1372, or Tet. Lett., 2002, 43, 1161.

Schemes 1 - 4 illustrate the conversion of phosphonate esters and phosphonic acids into carboalkoxy-substituted phosphorobisamidates (Scheme 1), phosphoroamidates (Scheme 2), phosphonate monoesters (Scheme 3) and phosphonate diesters, (Scheme 4).

Scheme 1 illustrates various methods for the conversion of phosphonate diesters **1.1** into phosphorobisamidates **1.5**. The diester **1.1**, prepared as described previously, is hydrolyzed, either to the monoester **1.2** or to the phosphonic acid **1.6**. The methods employed for these transformations are described above. The monoester **1.2** is converted into the monoamidate **1.3** by reaction with an aminoester **1.9**, in which the group  $R^2$  is H or alkyl, the group  $R^4$  is an alkylene moiety such as, for example,  $\text{CHCH}_3$ ,  $\text{CHPr}^I$ ,  $\text{CH}(\text{CH}_2\text{Ph})$ ,  $\text{CH}_2\text{CH}(\text{CH}_3)$  and the like, or a group present in natural or modified aminoacids, and the group  $R^5$  is alkyl. The reactants are combined in the presence of a coupling agent such as a carbodiimide, for example dicyclohexyl carbodiimide, as described in J. Am. Chem. Soc., 1957, 79, 3575, optionally in the presence of an activating agent such as hydroxybenztriazole, to yield the amidate product **1.3**. The amidate-forming reaction is also effected in the presence of coupling agents such as BOP, as described in J. Org. Chem., 1995, 60, 5214, Aldrithiol, PYBOP and similar coupling agents used for the preparation of amides and esters.

Alternatively, the reactants **1.2** and **1.9** are transformed into the monoamidate **1.3** by means of a Mitsunobu reaction. The preparation of amidates by means of the Mitsunobu reaction is described in J. Med. Chem., 1995, 38, 2742. Equimolar amounts of the reactants are

combined in an inert solvent such as tetrahydrofuran in the presence of a triaryl phosphine and a dialkyl azodicarboxylate. The thus-obtained monoamidate ester **1.3** is then transformed into amidate phosphonic acid **1.4**. The conditions used for the hydrolysis reaction depend on the nature of the  $R^1$  group, as described previously. The phosphonic acid amidate **1.4** is then reacted with an aminoester **1.9**, as described above, to yield the bisamidate product **1.5**, in which the amino substituents are the same or different.

An example of this procedure is shown in Scheme 1, Example 1. In this procedure, a dibenzyl phosphonate **1.14** is reacted with diazabicyclooctane (DABCO) in toluene at reflux, as described in J. Org. Chem., 1995, 60, 2946, to afford the monobenzyl phosphonate **1.15**. The product is then reacted with equimolar amounts of ethyl alaninate **1.16** and dicyclohexyl carbodiimide in pyridine, to yield the amidate product **1.17**. The benzyl group is then removed, for example by hydrogenolysis over a palladium catalyst, to give the monoacid product **1.18**. This compound is then reacted in a Mitsunobu reaction with ethyl leucinate **1.19**, triphenyl phosphine and diethylazodicarboxylate, as described in J. Med. Chem., 1995, 38, 2742, to produce the bisamidate product **1.20**.

Using the above procedures, but employing, in place of ethyl leucinate **1.19** or ethyl alaninate **1.16**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

Alternatively, the phosphonic acid **1.6** is converted into the bisamidate **1.5** by use of the coupling reactions described above. The reaction is performed in one step, in which case the nitrogen-related substituents present in the product **1.5** are the same, or in two steps, in which case the nitrogen-related substituents can be different.

An example of the method is shown in Scheme 1, Example 2. In this procedure, a phosphonic acid **1.6** is reacted in pyridine solution with excess ethyl phenylalaninate **1.21** and dicyclohexylcarbodiimide, for example as described in J. Chem. Soc., Chem. Comm., 1991, 1063, to give the bisamidate product **1.22**.

Using the above procedures, but employing, in place of ethyl phenylalaninate, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

As a further alternative, the phosphonic acid **1.6** is converted into the mono or bis-activated derivative **1.7**, in which Lv is a leaving group such as chloro, imidazolyl, triisopropylbenzenesulfonyloxy etc. The conversion of phosphonic acids into chlorides **1.7** (Lv = Cl) is effected by reaction with thionyl chloride or oxalyl chloride and the like, as described in *Organic Phosphorus Compounds*, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976, p. 17. The conversion of phosphonic acids into monoimidazolides **1.7** (Lv = imidazolyl) is described in *J. Med. Chem.*, 2002, 45, 1284 and in *J. Chem. Soc. Chem. Comm.*, 1991, 312. Alternatively, the phosphonic acid is activated by reaction with triisopropylbenzenesulfonyl chloride, as described in *Nucleosides and Nucleotides*, 2000, 10, 1885. The activated product is then reacted with the aminoester **1.9**, in the presence of a base, to give the bisamidate **1.5**. The reaction is performed in one step, in which case the nitrogen substituents present in the product **1.5** are the same, or in two steps, via the intermediate **1.11**, in which case the nitrogen substituents can be different.

Examples of these methods are shown in Scheme 1, Examples 3 and 5. In the procedure illustrated in Scheme 1, Example 3, a phosphonic acid **1.6** is reacted with ten molar equivalents of thionyl chloride, as described in *Zh. Obschei Khim.*, 1958, 28, 1063, to give the dichloro compound **1.23**. The product is then reacted at reflux temperature in a polar aprotic solvent such as acetonitrile, and in the presence of a base such as triethylamine, with butyl serinate **1.24** to afford the bisamidate product **1.25**.

Using the above procedures, but employing, in place of butyl serinate **1.24**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

In the procedure illustrated in Scheme 1, Example 5, the phosphonic acid **1.6** is reacted, as described in *J. Chem. Soc. Chem. Comm.*, 1991, 312, with carbonyl diimidazole to give the imidazolide **1.32**. The product is then reacted in acetonitrile solution at ambient temperature, with one molar equivalent of ethyl alaninate **1.33** to yield the monodisplacement product **1.34**. The latter compound is then reacted with carbonyl diimidazole to produce the activated intermediate **1.35**, and the product is then reacted, under the same conditions, with ethyl N-methylalaninate **1.33a** to give the bisamidate product **1.36**.

Using the above procedures, but employing, in place of ethyl alaninate **1.33** or ethyl N-methylalaninate **1.33a**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

The intermediate monoamidate **1.3** is also prepared from the monoester **1.2** by first converting the monoester into the activated derivative **1.8** in which Lv is a leaving group such as halo, imidazolyl etc, using the procedures described above. The product **1.8** is then reacted with an aminoester **1.9** in the presence of a base such as pyridine, to give an intermediate monoamidate product **1.3**. The latter compound is then converted, by removal of the R<sup>1</sup> group and coupling of the product with the aminoester **1.9**, as described above, into the bisamidate **1.5**.

An example of this procedure, in which the phosphonic acid is activated by conversion to the chloro derivative **1.26**, is shown in Scheme 1, Example 4. In this procedure, the phosphonic monobenzyl ester **1.15** is reacted, in dichloromethane, with thionyl chloride, as described in Tet. Let., 1994, 35, 4097, to afford the phosphoryl chloride **1.26**. The product is then reacted in acetonitrile solution at ambient temperature with one molar equivalent of ethyl 3-amino-2-methylpropionate **1.27** to yield the monoamidate product **1.28**. The latter compound is hydrogenated in ethyl acetate over a 5% palladium on carbon catalyst to produce the monoacid product **1.29**. The product is subjected to a Mitsunobu coupling procedure, with equimolar amounts of butyl alaninate **1.30**, triphenyl phosphine, diethylazodicarboxylate and triethylamine in tetrahydrofuran, to give the bisamidate product **1.31**.

Using the above procedures, but employing, in place of ethyl 3-amino-2-methylpropionate **1.27** or butyl alaninate **1.30**, different aminoesters **1.9**, the corresponding products **1.5** are obtained.

The activated phosphonic acid derivative **1.7** is also converted into the bisamidate **1.5** via the diamino compound **1.10**. The conversion of activated phosphonic acid derivatives such as phosphoryl chlorides into the corresponding amino analogs **1.10**, by reaction with ammonia, is described in Organic Phosphorus Compounds, G. M. Kosolapoff, L. Maeir, eds, Wiley, 1976. The diamino compound **1.10** is then reacted at elevated temperature with a haloester

**1.12**, in a polar organic solvent such as dimethylformamide, in the presence of a base such as dimethylaminopyridine or potassium carbonate, to yield the bisamidate **1.5**.

An example of this procedure is shown in Scheme 1, Example 6. In this method, a dichlorophosphonate **1.23** is reacted with ammonia to afford the diamide **1.37**. The reaction is performed in aqueous, aqueous alcoholic or alcoholic solution, at reflux temperature. The resulting diamino compound is then reacted with two molar equivalents of ethyl 2-bromo-3-methylbutyrate **1.38**, in a polar organic solvent such as N-methylpyrrolidinone at ca. 150°C, in the presence of a base such as potassium carbonate, and optionally in the presence of a catalytic amount of potassium iodide, to afford the bisamidate product **1.39**.

Using the above procedures, but employing, in place of ethyl 2-bromo-3-methylbutyrate **1.38**, different haloesters **1.12** the corresponding products **1.5** are obtained.

The procedures shown in Scheme 1 are also applicable to the preparation of bisamidates in which the aminoester moiety incorporates different functional groups. Scheme 1, Example 7 illustrates the preparation of bisamidates derived from tyrosine. In this procedure, the monoimidazolidine **1.32** is reacted with propyl tyrosinate **1.40**, as described in Example 5, to yield the monoamidate **1.41**. The product is reacted with carbonyl diimidazole to give the imidazolidine **1.42**, and this material is reacted with a further molar equivalent of propyl tyrosinate to produce the bisamidate product **1.43**.

Using the above procedures, but employing, in place of propyl tyrosinate **1.40**, different aminoesters **1.9**, the corresponding products **1.5** are obtained. The aminoesters employed in the two stages of the above procedure can be the same or different, so that bisamidates with the same or different amino substituents are prepared.

Scheme 2 illustrates methods for the preparation of phosphonate monoamidates.

In one procedure, a phosphonate monoester **1.1** is converted, as described in Scheme 1, into the activated derivative **1.8**. This compound is then reacted, as described above, with an aminoester **1.9**, in the presence of a base, to afford the monoamidate product **2.1**.

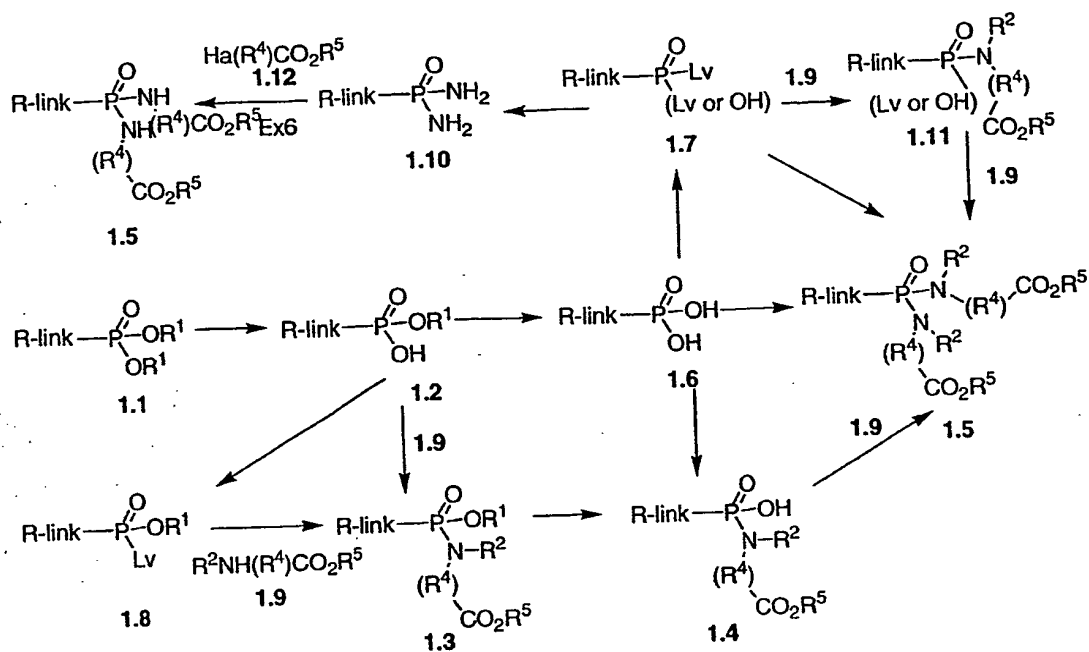
The procedure is illustrated in Scheme 2, Example 1. In this method, a monophenyl phosphonate **2.7** is reacted with, for example, thionyl chloride, as described in J. Gen. Chem.

USSR., 1983, 32, 367, to give the chloro product 2.8. The product is then reacted, as described in Scheme 1, with ethyl alaninate 2.9, to yield the amidate 2.10.

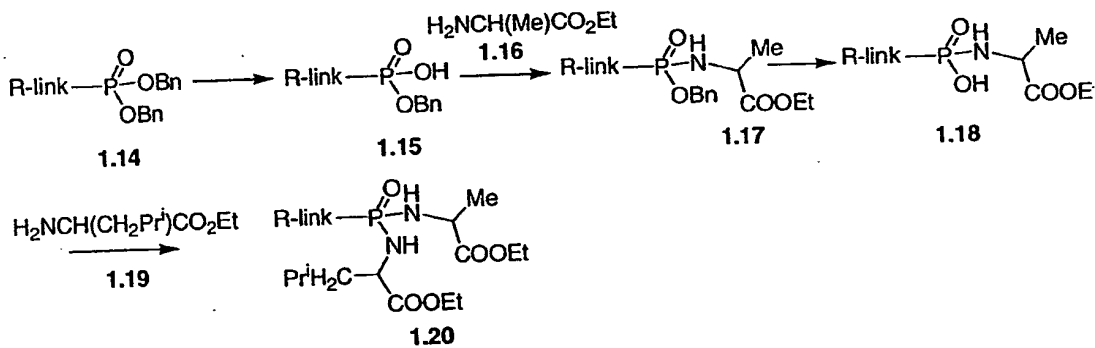
Using the above procedures, but employing, in place of ethyl alaninate 2.9, different aminoesters 1.9, the corresponding products 2.1 are obtained.

Alternatively, the phosphonate monoester 1.1 is coupled, as described in Scheme 1, with an aminoester 1.9 to produce the amidate 2.1. If necessary, the R<sup>1</sup> substituent is then altered, by initial cleavage to afford the phosphonic acid 2.2. The procedures for this transformation depend on the nature of the R<sup>1</sup> group, and are described above. The phosphonic acid is then transformed into the ester amidate product 2.3, by reaction with the hydroxy compound R<sup>3</sup>OH, in which the group R<sup>3</sup> is aryl, heteroaryl, alkyl, cycloalkyl, haloalkyl etc, using the same coupling procedures (carbodiimide, Aldrithiol-2, PYBOP, Mitsunobu reaction etc) described in Scheme 1 for the coupling of amines and phosphonic acids.

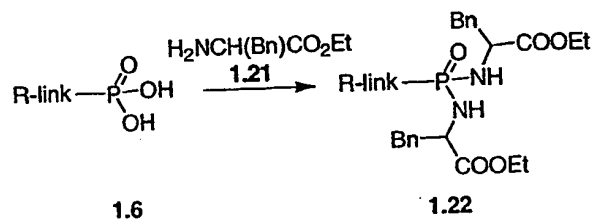
Scheme 1



Scheme 1 Example 1

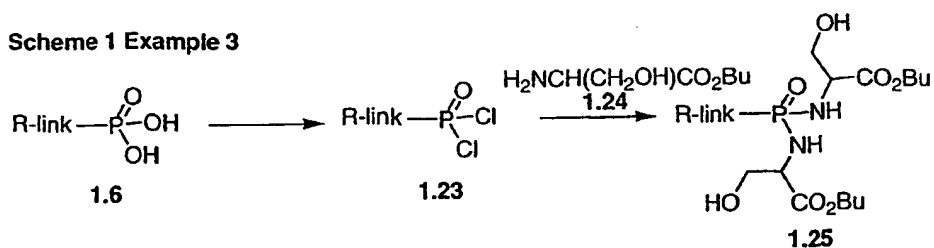


Scheme 1 Example 2

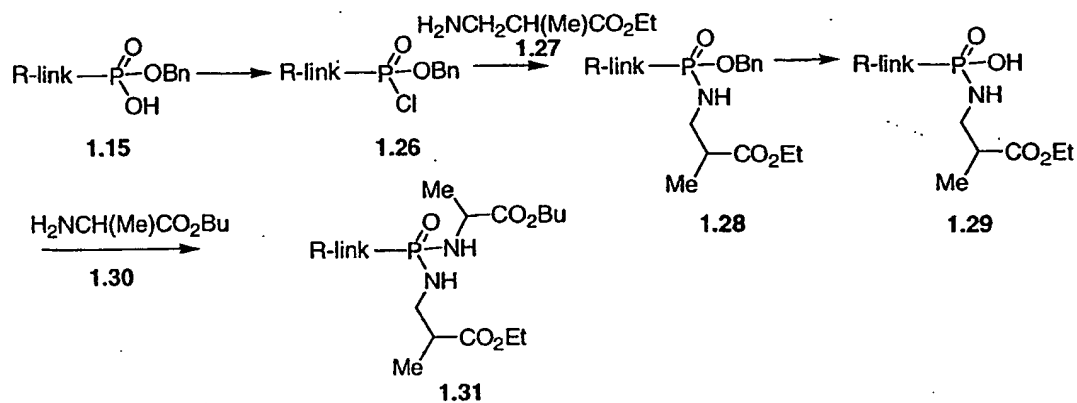




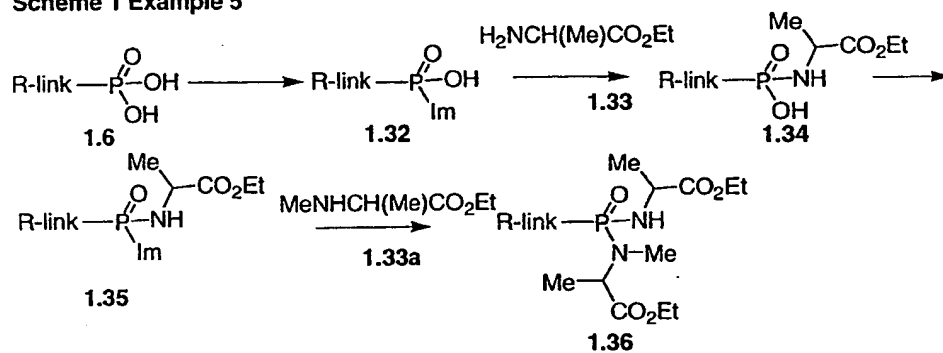
## Scheme 1 Example 3



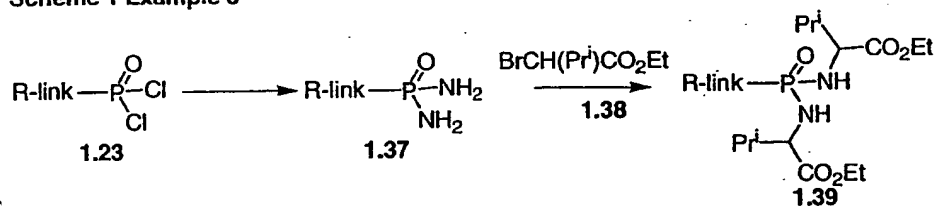
## Scheme 1 Example 4



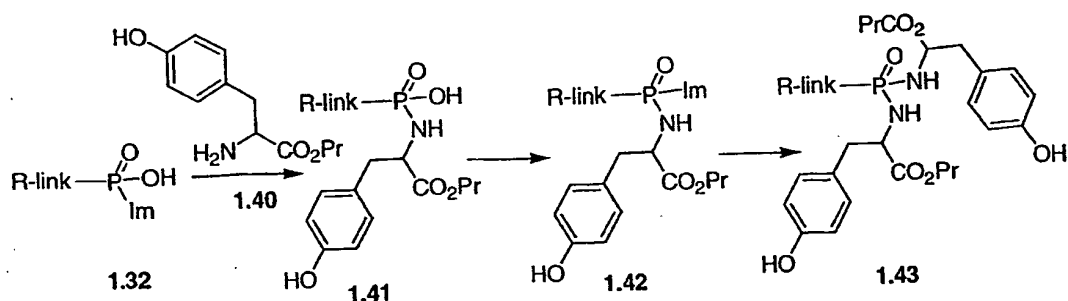
## Scheme 1 Example 5



## Scheme 1 Example 6



Scheme 1 Example 7



Examples of this method are shown in Scheme 2, Examples and 2 and 3. In the sequence shown in Example 2, a monobenzyl phosphonate **2.11** is transformed by reaction with ethyl alaninate, using one of the methods described above, into the monoamidate **2.12**. The benzyl group is then removed by catalytic hydrogenation in ethyl acetate solution over a 5% palladium on carbon catalyst, to afford the phosphonic acid amidate **2.13**. The product is then reacted in dichloromethane solution at ambient temperature with equimolar amounts of 1-(dimethylaminopropyl)-3-ethylcarbodiimide and trifluoroethanol **2.14**, for example as described in Tet. Lett., 2001, 42, 8841, to yield the amidate ester **2.15**.

In the sequence shown in Scheme 2, Example 3, the monoamidate **2.13** is coupled, in tetrahydrofuran solution at ambient temperature, with equimolar amounts of dicyclohexyl carbodiimide and 4-hydroxy-N-methylpiperidine **2.16**, to produce the amidate ester product **2.17**.

Using the above procedures, but employing, in place of the ethyl alaninate product **2.12** different monoacids **2.2**, and in place of trifluoroethanol **2.14** or 4-hydroxy-N-methylpiperidine **2.16**, different hydroxy compounds R<sup>3</sup>OH, the corresponding products **2.3** are obtained.

Alternatively, the activated phosphonate ester **1.8** is reacted with ammonia to yield the amidate **2.4**. The product is then reacted, as described in Scheme 1, with a haloester **2.5**, in the presence of a base, to produce the amidate product **2.6**. If appropriate, the nature of the R<sup>1</sup> group is changed, using the procedures described above, to give the product **2.3**. The method is illustrated in Scheme 2, Example 4. In this sequence, the monophenyl phosphoryl

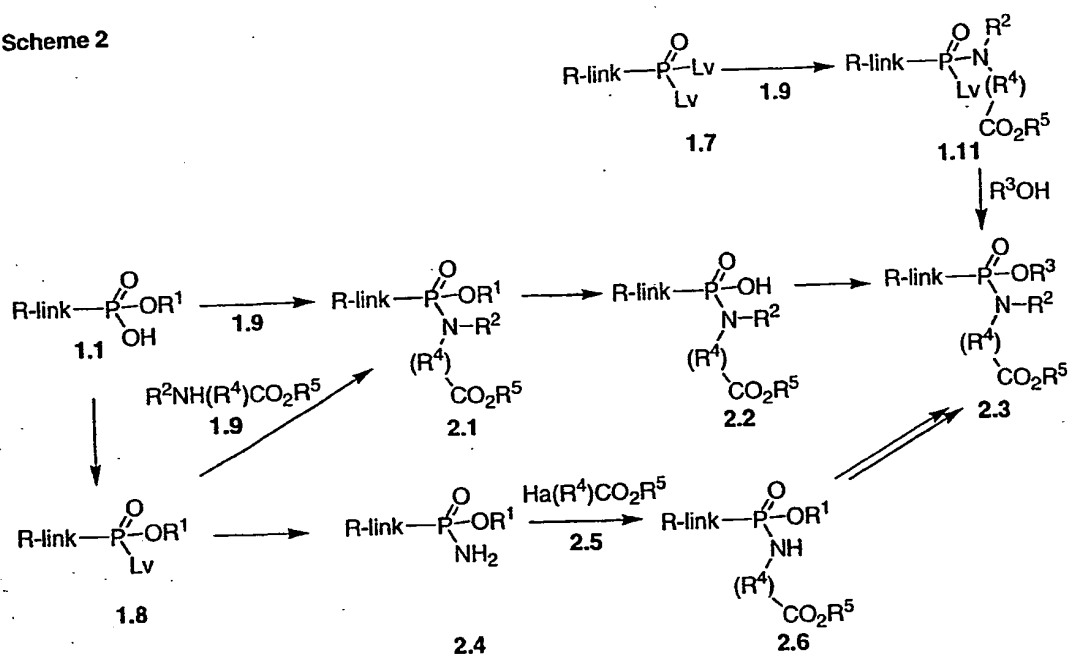
chloride **2.18** is reacted, as described in Scheme 1, with ammonia, to yield the amino product **2.19**. This material is then reacted in N-methylpyrrolidinone solution at 170°C with butyl 2-bromo-3-phenylpropionate **2.20** and potassium carbonate, to afford the amidate product **2.21**. Using these procedures, but employing, in place of butyl 2-bromo-3-phenylpropionate **2.20**, different haloesters **2.5**, the corresponding products **2.6** are obtained.

The monoamidate products **2.3** are also prepared from the doubly activated phosphonate derivatives **1.7**. In this procedure, examples of which are described in Synlett., 1998, 1, 73, the intermediate **1.7** is reacted with a limited amount of the aminoester **1.9** to give the mono-displacement product **1.11**. The latter compound is then reacted with the hydroxy compound  $R^3OH$  in a polar organic solvent such as dimethylformamide, in the presence of a base such as diisopropylethylamine, to yield the monoamidate ester **2.3**.

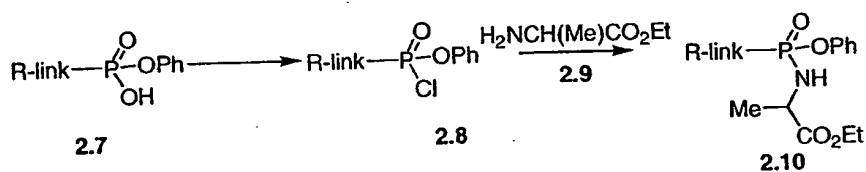
The method is illustrated in Scheme 2, Example 5. In this method, the phosphoryl dichloride **2.22** is reacted in dichloromethane solution with one molar equivalent of ethyl N-methyl tyrosinate **2.23** and dimethylaminopyridine, to generate the monoamidate **2.24**. The product is then reacted with phenol **2.25** in dimethylformamide containing potassium carbonate, to yield the ester amidate product **2.26**.

Using these procedures, but employing, in place of ethyl N-methyl tyrosinate **2.23** or phenol **2.25**, the aminoesters **1.9** and/or the hydroxy compounds  $R^3OH$ , the corresponding products **2.3** are obtained.

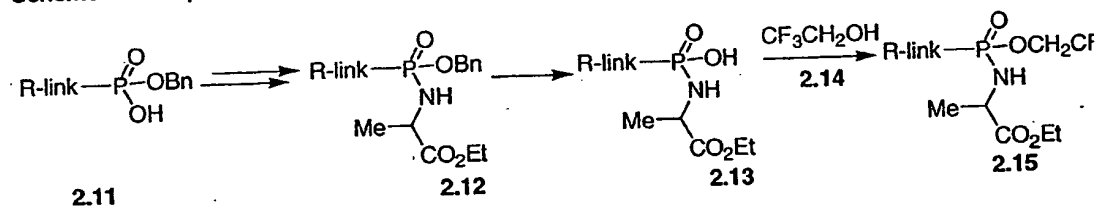
Scheme 2



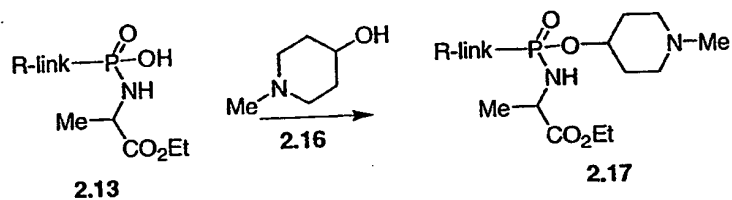
Scheme 2 Example 1



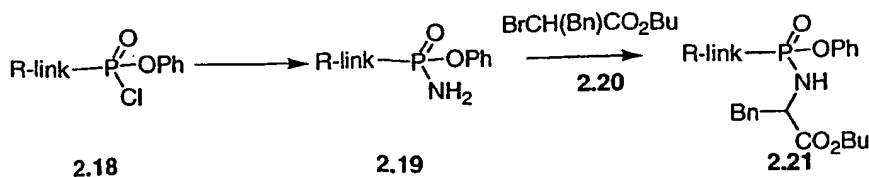
Scheme 2 Example 2



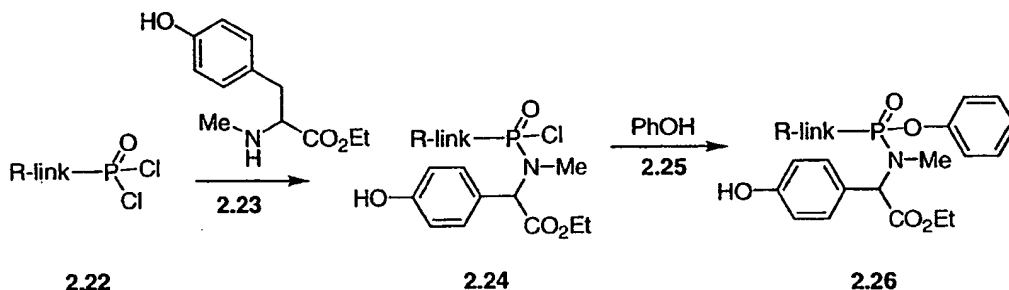
Scheme 2 Example 3



## Scheme 2 Example 4



## Scheme 2 Example 5



Scheme 3 illustrates methods for the preparation of carboalkoxy-substituted phosphonate diesters in which one of the ester groups incorporates a carboalkoxy substituent.

In one procedure, a phosphonate monoester 1.1, prepared as described above, is coupled, using one of the methods described above, with a hydroxyester 3.1, in which the groups  $\text{R}^4$  and  $\text{R}^5$  are as described in Scheme 1. For example, equimolar amounts of the reactants are coupled in the presence of a carbodiimide such as dicyclohexyl carbodiimide, as described in Aust. J. Chem., 1963, 609, optionally in the presence of dimethylaminopyridine, as described in Tet., 1999, 55, 12997. The reaction is conducted in an inert solvent at ambient temperature.

The procedure is illustrated in Scheme 3, Example 1. In this method, a monophenyl phosphonate 3.9 is coupled, in dichloromethane solution in the presence of dicyclohexyl carbodiimide, with ethyl 3-hydroxy-2-methylpropionate 3.10 to yield the phosphonate mixed diester 3.11.

Using this procedure, but employing, in place of ethyl 3-hydroxy-2-methylpropionate 3.10, different hydroxyesters 3.1, the corresponding products 3.2 are obtained.

The conversion of a phosphonate monoester **1.1** into a mixed diester **3.2** is also accomplished by means of a Mitsunobu coupling reaction with the hydroxyester **3.1**, as described in Org. Lett., 2001, 643. In this method, the reactants **1.1** and **3.1** are combined in a polar solvent such as tetrahydrofuran, in the presence of a triarylphosphine and a dialkyl azodicarboxylate, to give the mixed diester **3.2**. The R<sup>1</sup> substituent is varied by cleavage, using the methods described previously, to afford the monoacid product **3.3**. The product is then coupled, for example using methods described above, with the hydroxy compound R<sup>3</sup>OH, to give the diester product **3.4**.

The procedure is illustrated in Scheme 3, Example 2. In this method, a monoallyl phosphonate **3.12** is coupled in tetrahydrofuran solution, in the presence of triphenylphosphine and diethylazodicarboxylate, with ethyl lactate **3.13** to give the mixed diester **3.14**. The product is reacted with tris(triphenylphosphine) rhodium chloride (Wilkinson catalyst) in acetonitrile, as described previously, to remove the allyl group and produce the monoacid product **3.15**. The latter compound is then coupled, in pyridine solution at ambient temperature, in the presence of dicyclohexyl carbodiimide, with one molar equivalent of 3-hydroxypyridine **3.16** to yield the mixed diester **3.17**.

Using the above procedures, but employing, in place of the ethyl lactate **3.13** or 3-hydroxypyridine, a different hydroxyester **3.1** and/or a different hydroxy compound R<sup>3</sup>OH, the corresponding products **3.4** are obtained.

The mixed diesters **3.2** are also obtained from the monoesters **1.1** via the intermediacy of the activated monoesters **3.5**. In this procedure, the monoester **1.1** is converted into the activated compound **3.5** by reaction with, for example, phosphorus pentachloride, as described in J. Org. Chem., 2001, 66, 329, or with thionyl chloride or oxalyl chloride (Lv = Cl), or with triisopropylbenzenesulfonyl chloride in pyridine, as described in Nucleosides and Nucleotides, 2000, 19, 1885, or with carbonyl diimidazole, as described in J. Med. Chem., 2002, 45, 1284. The resultant activated monoester is then reacted with the hydroxyester **3.1**, as described above, to yield the mixed diester **3.2**.

The procedure is illustrated in Scheme 3, Example 3. In this sequence, a monophenyl phosphonate **3.9** is reacted, in acetonitrile solution at 70°C, with ten equivalents of thionyl

chloride, so as to produce the phosphoryl chloride 3.19. The product is then reacted with ethyl 4-carbamoyl-2-hydroxybutyrate 3.20 in dichloromethane containing triethylamine, to give the mixed diester 3.21.

Using the above procedures, but employing, in place of ethyl 4-carbamoyl-2-hydroxybutyrate 3.20, different hydroxyesters 3.1, the corresponding products 3.2 are obtained.

The mixed phosphonate diesters are also obtained by an alternative route for incorporation of the  $R^3O$  group into intermediates 3.3 in which the hydroxyester moiety is already incorporated. In this procedure, the monoacid intermediate 3.3 is converted into the activated derivative 3.6 in which  $Lv$  is a leaving group such as chloro, imidazole, and the like, as previously described. The activated intermediate is then reacted with the hydroxy compound  $R^3OH$ , in the presence of a base, to yield the mixed diester product 3.4.

The method is illustrated in Scheme 3, Example 4. In this sequence, the phosphonate monoacid 3.22 is reacted with trichloromethanesulfonyl chloride in tetrahydrofuran containing collidine, as described in J. Med. Chem., 1995, 38, 4648, to produce the trichloromethanesulfonyloxy product 3.23. This compound is reacted with 3-(morpholinomethyl)phenol 3.24 in dichloromethane containing triethylamine, to yield the mixed diester product 3.25.

Using the above procedures, but employing, in place of with 3-(morpholinomethyl)phenol 3.24, different carbinols  $R^3OH$ , the corresponding products 3.4 are obtained.

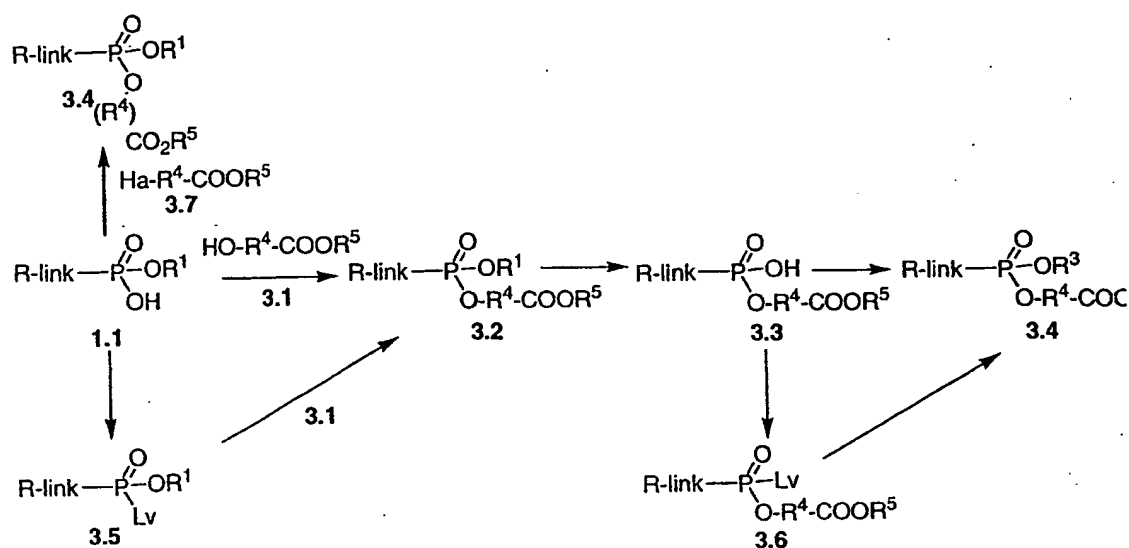
The phosphonate esters 3.4 are also obtained by means of alkylation reactions performed on the monoesters 1.1. The reaction between the monoacid 1.1 and the haloester 3.7 is performed in a polar solvent in the presence of a base such as diisopropylethylamine, as described in Anal. Chem., 1987, 59, 1056, or triethylamine, as described in J. Med. Chem., 1995, 38, 1372, or in a non-polar solvent such as benzene, in the presence of 18-crown-6, as described in Syn. Comm., 1995, 25, 3565.

The method is illustrated in Scheme 3, Example 5. In this procedure, the monoacid 3.26 is reacted with ethyl 2-bromo-3-phenylpropionate 3.27 and diisopropylethylamine in dimethylformamide at 80°C to afford the mixed diester product 3.28.

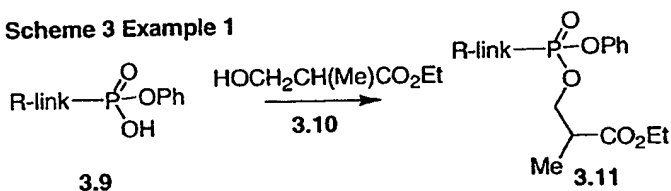
Using the above procedure, but employing, in place of ethyl 2-bromo-3-phenylpropionate 3.27, different haloesters 3.7, the corresponding products 3.4 are obtained.



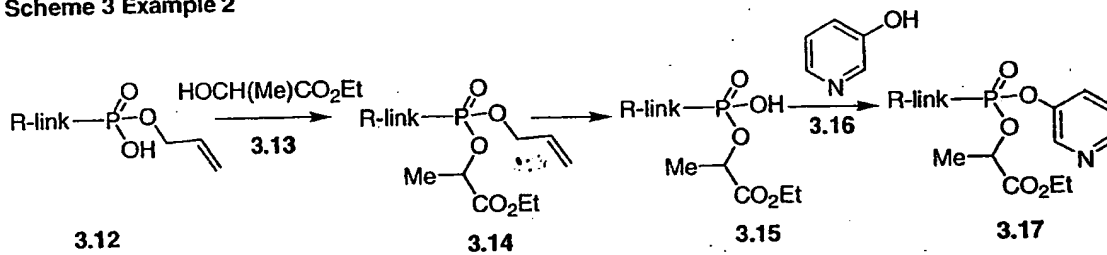
Scheme 3



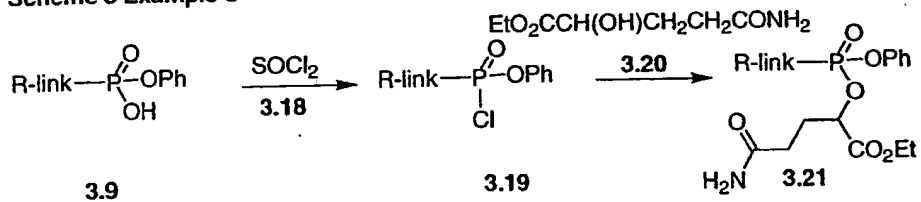
Scheme 3 Example 1



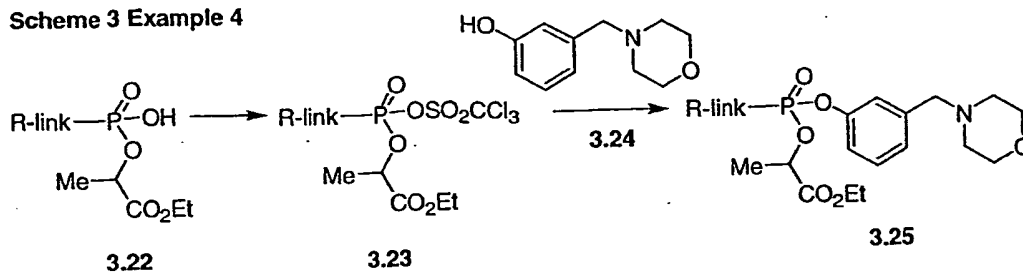
Scheme 3 Example 2



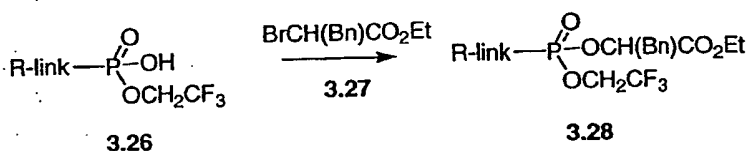
Scheme 3 Example 3



## Scheme 3 Example 4



## Scheme 3 Example 5



Scheme 4 illustrates methods for the preparation of phosphonate diesters in which both the ester substituents incorporate carboalkoxy groups.

The compounds are prepared directly or indirectly from the phosphonic acids 1.6. In one alternative, the phosphonic acid is coupled with the hydroxyester 4.2, using the conditions described previously in Schemes 1 - 3, such as coupling reactions using dicyclohexyl carbodiimide or similar reagents, or under the conditions of the Mitsunobu reaction, to afford the diester product 4.3 in which the ester substituents are identical.

This method is illustrated in Scheme 4, Example 1. In this procedure, the phosphonic acid 1.6 is reacted with three molar equivalents of butyl lactate 4.5 in the presence of Aldrithiol-2 and triphenyl phosphine in pyridine at ca. 70°C, to afford the diester 4.6.

Using the above procedure, but employing, in place of butyl lactate 4.5, different hydroxyesters 4.2, the corresponding products 4.3 are obtained.

Alternatively, the diesters 4.3 are obtained by alkylation of the phosphonic acid 1.6 with a haloester 4.1. The alkylation reaction is performed as described in Scheme 3 for the preparation of the esters 3.4.

This method is illustrated in Scheme 4, Example 2. In this procedure, the phosphonic acid 1.6 is reacted with excess ethyl 3-bromo-2-methylpropionate 4.7 and diisopropylethylamine in dimethylformamide at ca. 80°C, as described in Anal. Chem., 1987, 59, 1056, to produce the diester 4.8.

Using the above procedure, but employing, in place of ethyl 3-bromo-2-methylpropionate 4.7, different haloesters 4.1, the corresponding products 4.3 are obtained.

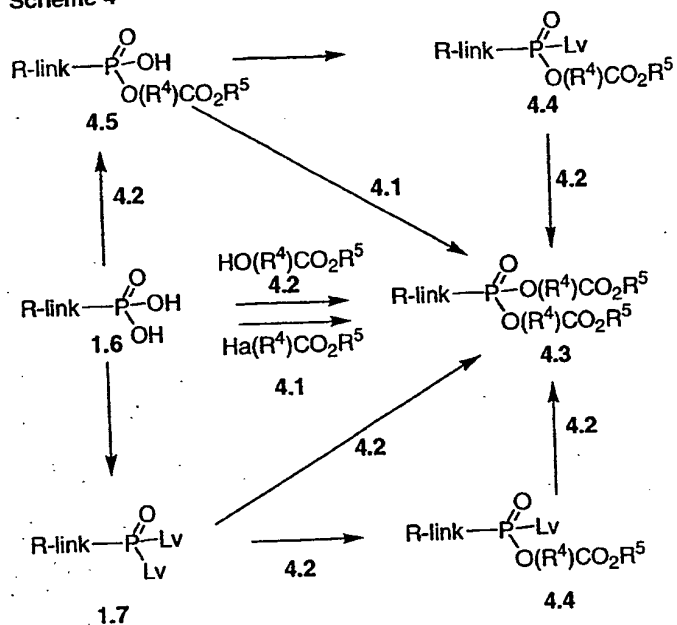
The diesters 4.3 are also obtained by displacement reactions of activated derivatives 1.7 of the phosphonic acid with the hydroxyesters 4.2. The displacement reaction is performed in a polar solvent in the presence of a suitable base, as described in Scheme 3. The displacement reaction is performed in the presence of an excess of the hydroxyester, to afford the diester product 4.3 in which the ester substituents are identical, or sequentially with limited amounts of different hydroxyesters, to prepare diesters 4.3 in which the ester substituents are different. The methods are illustrated in Scheme 4, Examples 3 and 4. As shown in Example 3, the phosphoryl dichloride 2.22 is reacted with three molar equivalents of ethyl 3-hydroxy-2-(hydroxymethyl)propionate 4.9 in tetrahydrofuran containing potassium carbonate, to obtain the diester product 4.10.

Using the above procedure, but employing, in place of ethyl 3-hydroxy-2-(hydroxymethyl)propionate 4.9, different hydroxyesters 4.2, the corresponding products 4.3 are obtained.

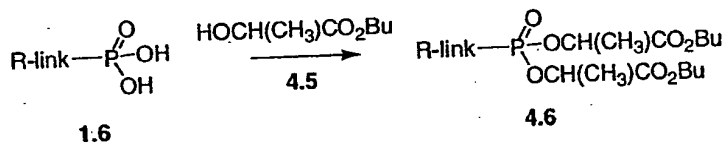
Scheme 4, Example 4 depicts the displacement reaction between equimolar amounts of the phosphoryl dichloride 2.22 and ethyl 2-methyl-3-hydroxypropionate 4.11, to yield the monoester product 4.12. The reaction is conducted in acetonitrile at 70°C in the presence of diisopropylethylamine. The product 4.12 is then reacted, under the same conditions, with one molar equivalent of ethyl lactate 4.13, to give the diester product 4.14.

Using the above procedures, but employing, in place of ethyl 2-methyl-3-hydroxypropionate 4.11 and ethyl lactate 4.13, sequential reactions with different hydroxyesters 4.2, the corresponding products 4.3 are obtained.

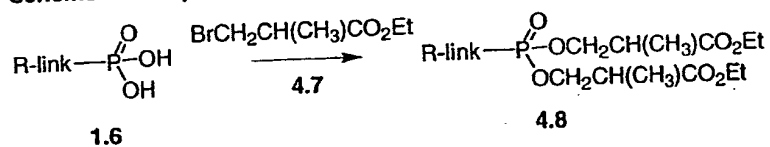
Scheme 4



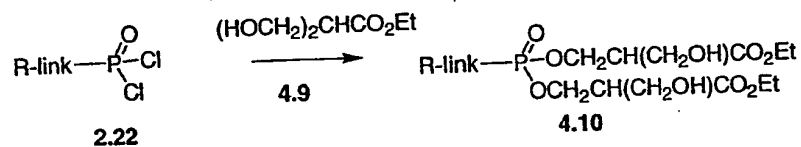
Scheme 4 Example 1



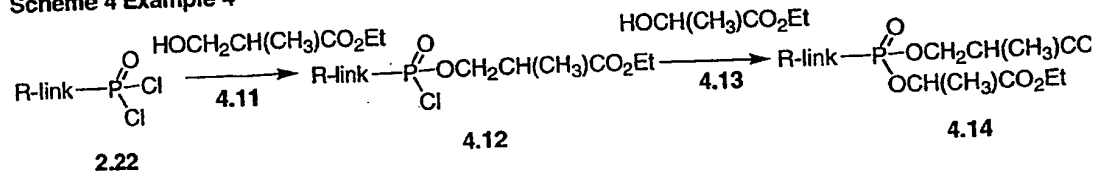
Scheme 4 Example 2



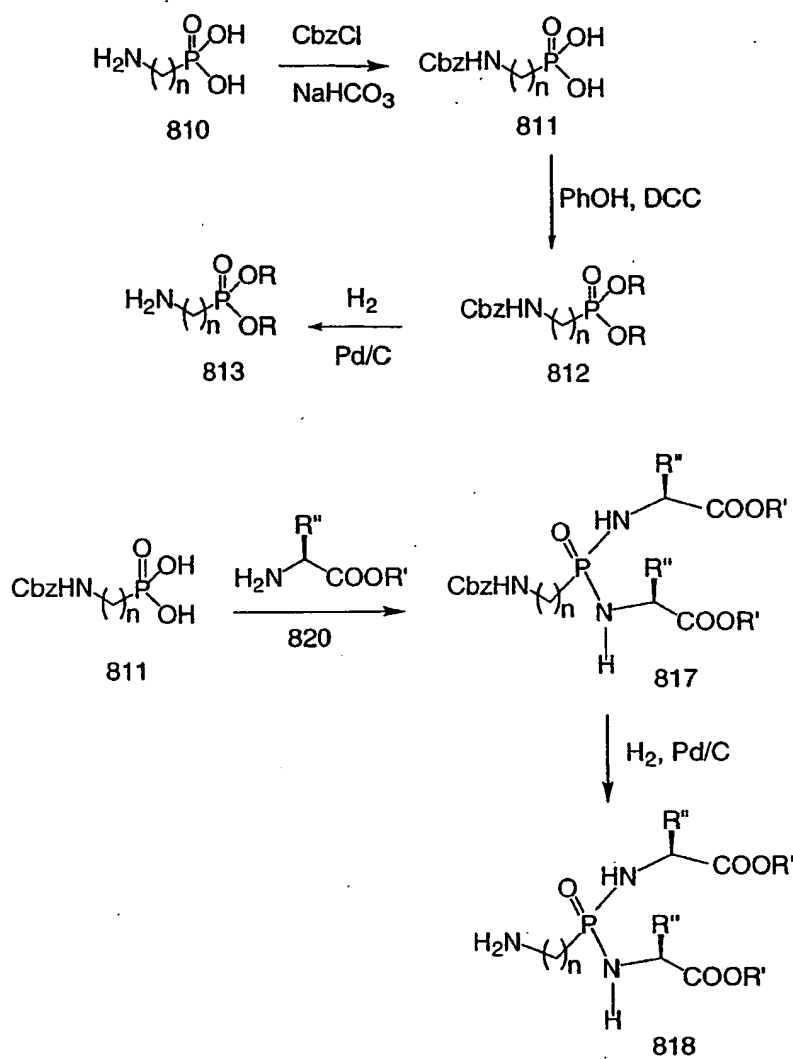
Scheme 4 Example 3

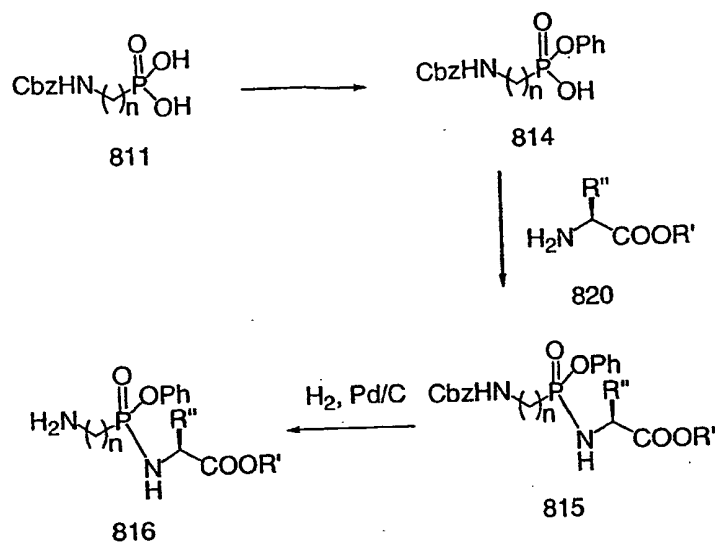


Scheme 4 Example 4



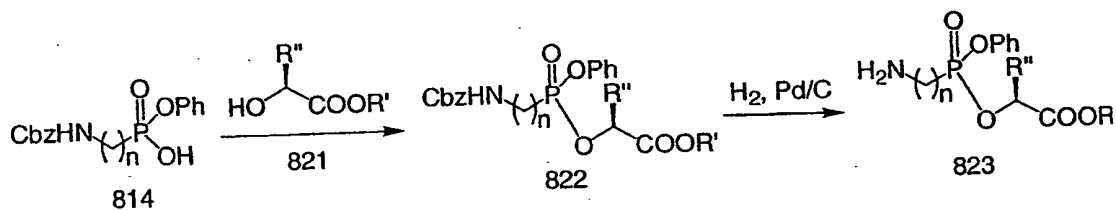
Scheme 1002



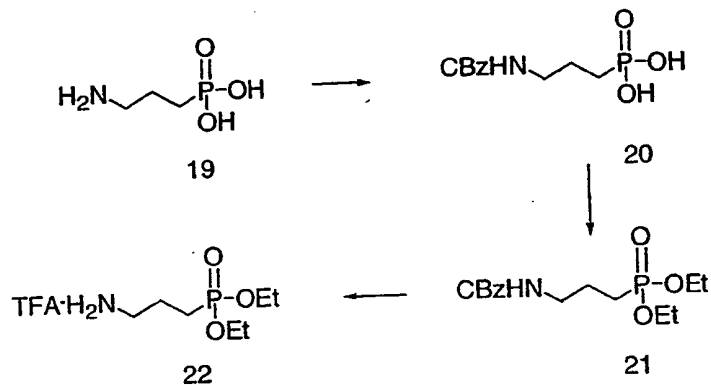


Following the similar procedures, replacement of amino acid esters 820 with lactates 821 (Scheme 1003) provides mono-phosphonic lactates 823. Lactates 823 are useful intermediates to form the phosphonate compounds of the invention.

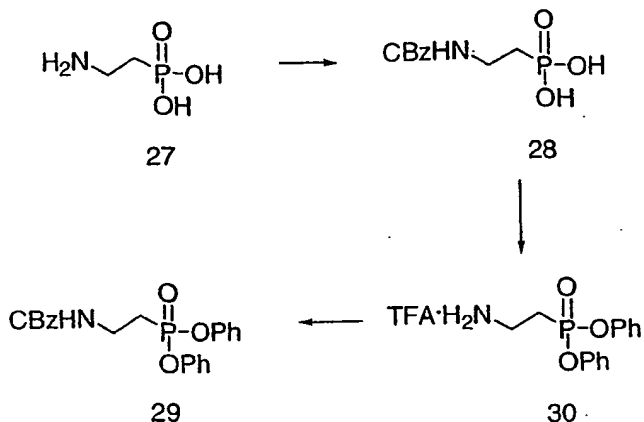
Scheme 1003



Scheme 1004



Scheme 1005

Example 1

To a solution of 2-aminoethylphosphonic acid (1.26 g, 10.1 mmol) in 2N NaOH (10.1 mL, 20.2 mmol) was added benzyl chloroformate (1.7 mL, 12.1 mmol). After the reaction mixture was stirred for 2 d at room temperature, the mixture was partitioned between Et<sub>2</sub>O and water. The aqueous phase was acidified with 6N HCl until pH = 2. The resulting colorless solid was dissolved in MeOH (75 mL) and treated with Dowex 50WX8-200 (7 g). After the mixture was stirred for 30 minutes, it was filtered and evaporated under reduced pressure to give carbamate **28** (2.37 g, 91%) as a colorless solid (Scheme 1005).

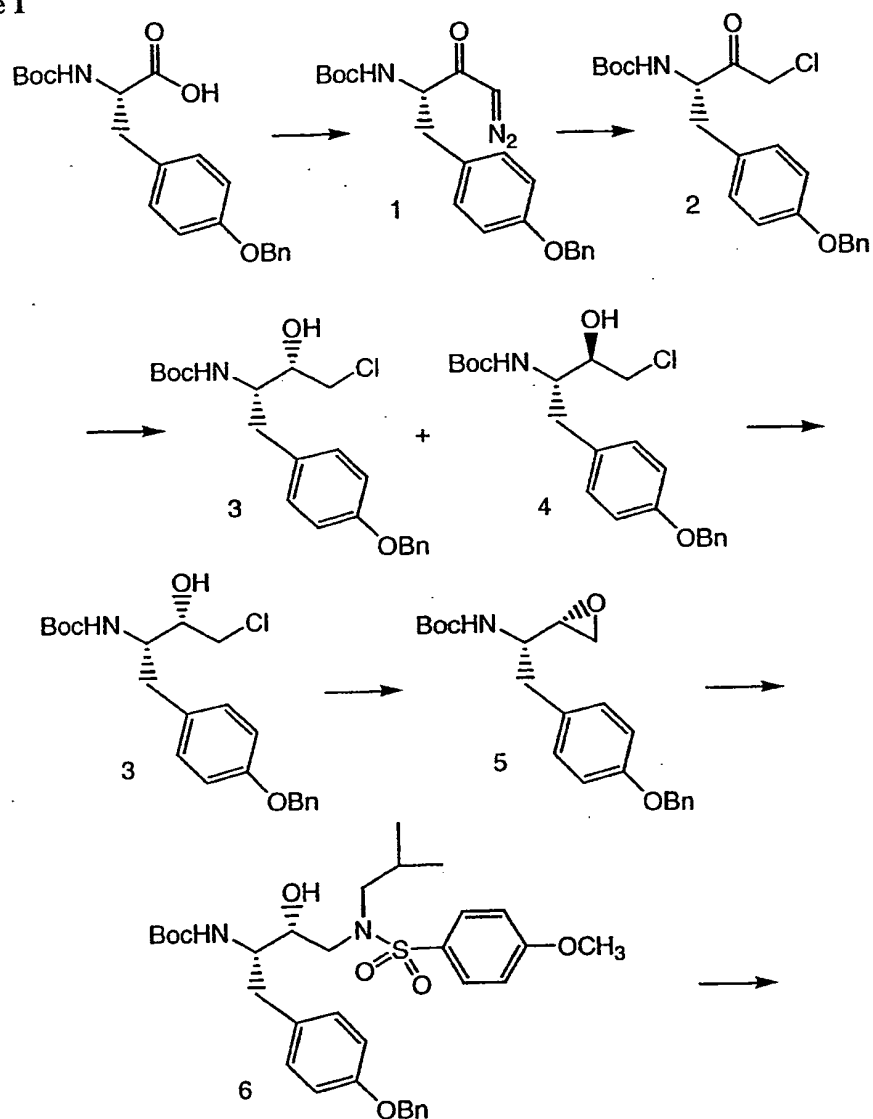
To a solution of carbamate **28** (2.35 g, 9.1 mmol) in pyridine (40 mL) was added phenol (8.53 g, 90.6 mmol) and 1,3-dicyclohexylcarbodiimide (7.47 g, 36.2 mmol). After the reaction mixture was warmed to 70°C and stirred for 5 h, the mixture was diluted with CH<sub>3</sub>CN and filtered. The filtrate was concentrated under reduced pressure and diluted with EtOAc. The organic phase was washed with sat. NH<sub>4</sub>Cl, sat. NaHCO<sub>3</sub>, and brine, then dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel twice (eluting 40-60% EtOAc/hexane) to give phosphonate **29** (2.13 g, 57%) as a colorless solid.

To a solution of phosphonate **29** (262 mg, 0.637 mmol) in iPrOH (5 mL) was added TFA (0.05 mL, 0.637 mmol) and 10% Pd/C (26 mg). After the reaction mixture was stirred under H<sub>2</sub> atmosphere (balloon) for 1 h, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give amine **30** (249 mg, 100%) as a colorless oil (Scheme 1005).

### Scheme Section A

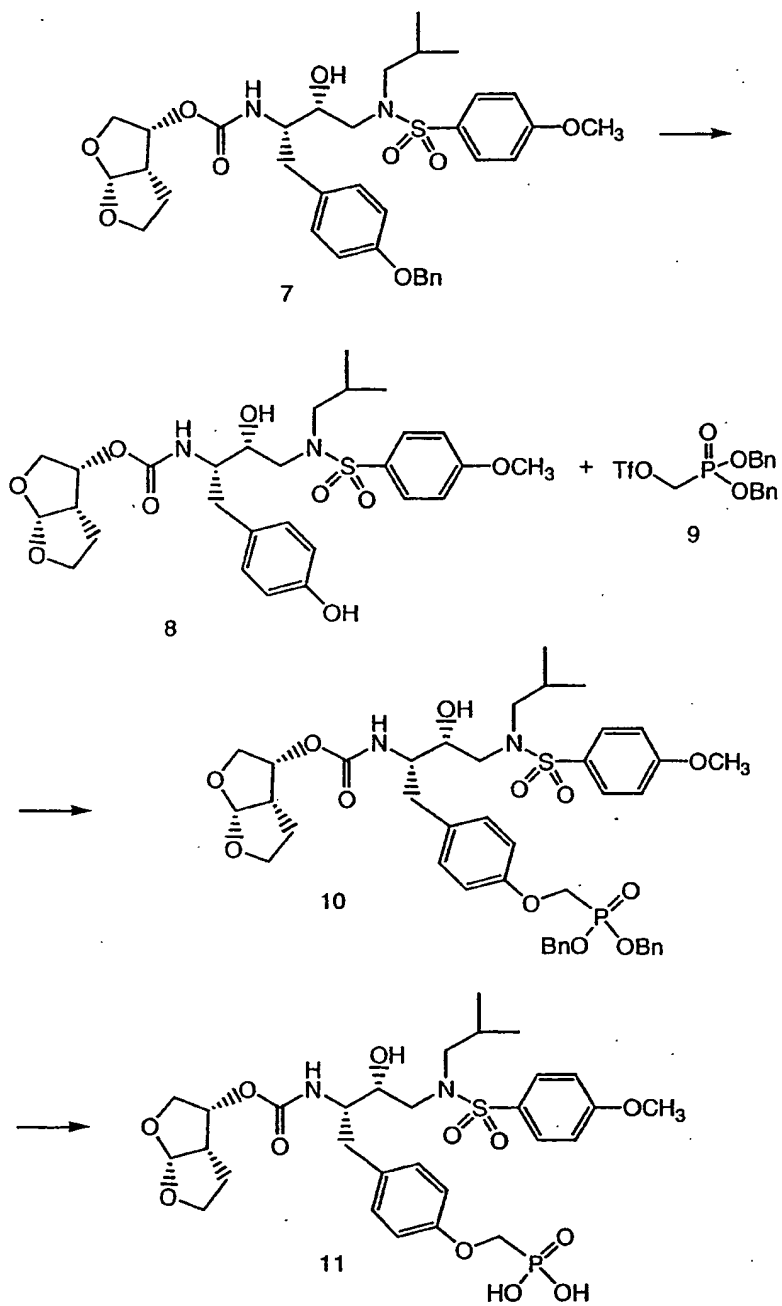
Exemplary methods of preparing the compounds of the invention are shown in **Schemes 1-7** below. A detailed description of the methods is found in the Experimental section below.

### Scheme 1

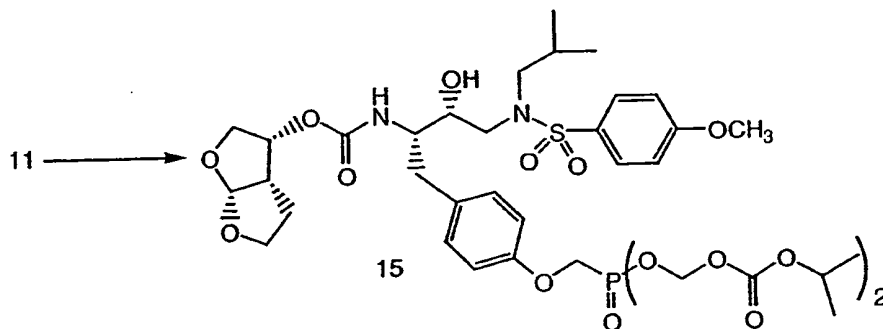
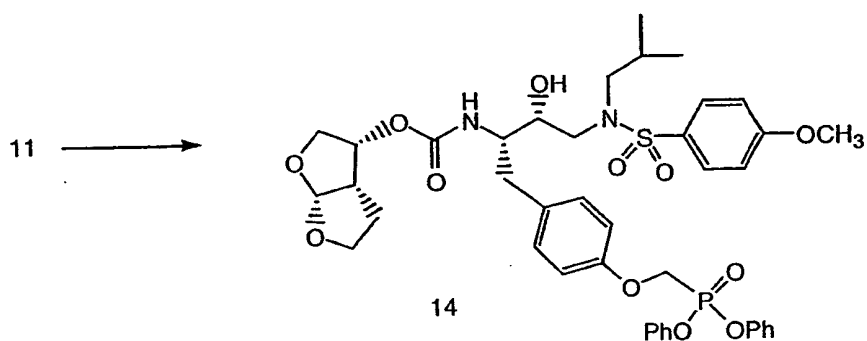
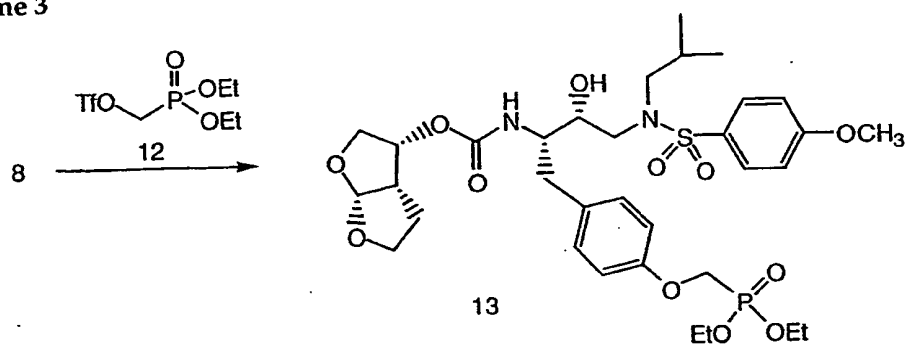




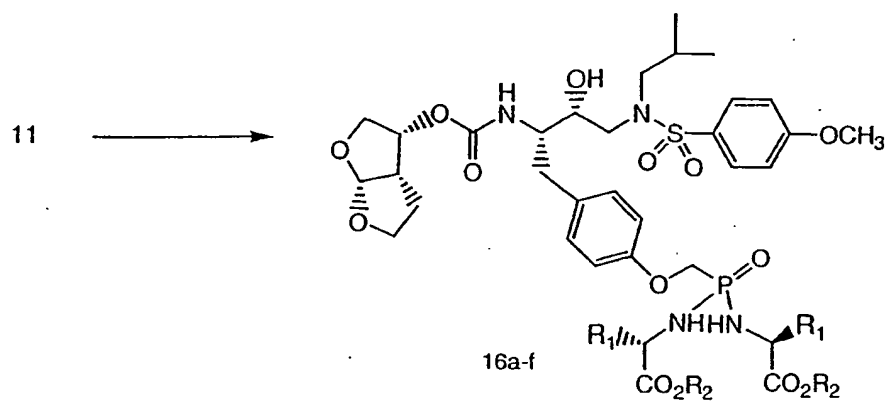
Scheme 2



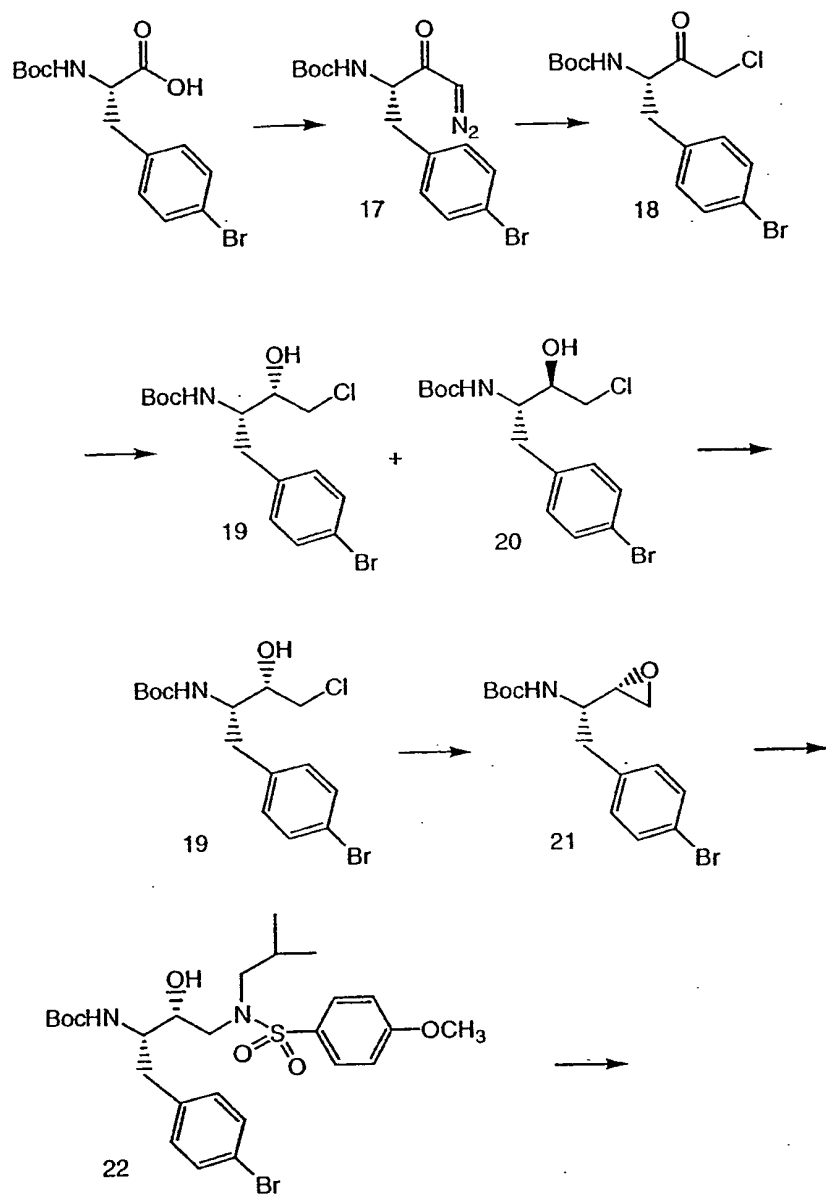
## Scheme 3



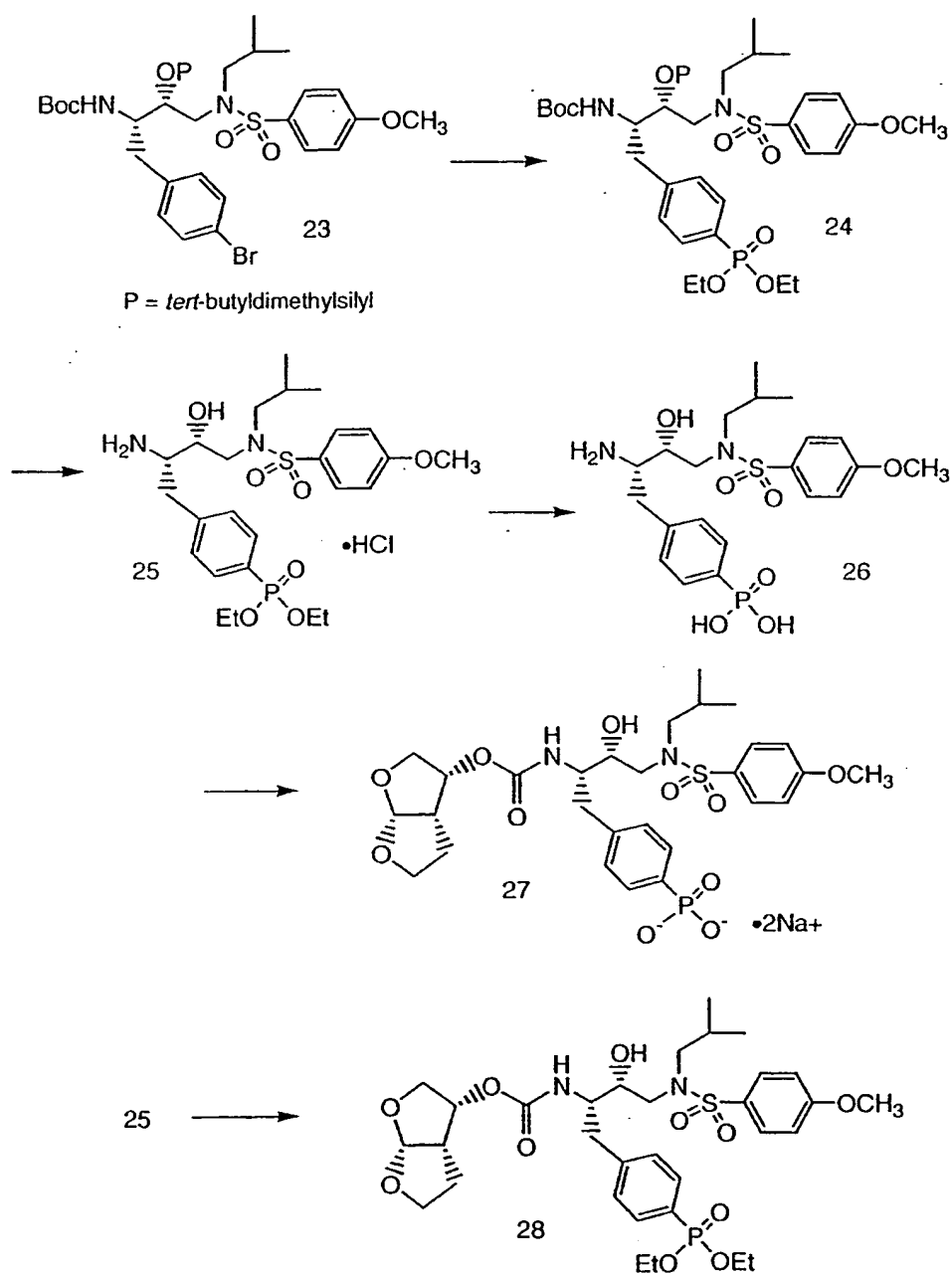
## Scheme 4



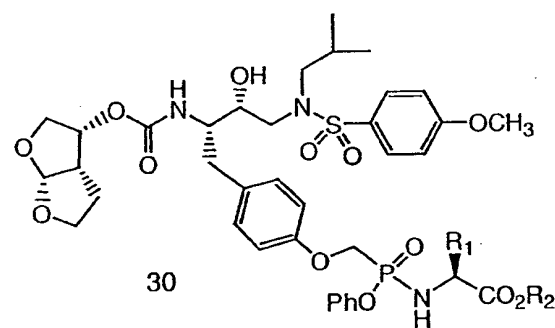
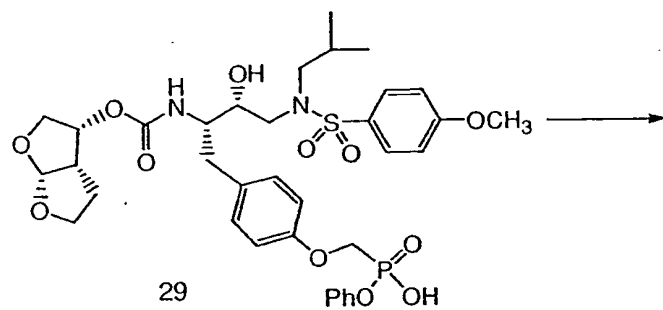
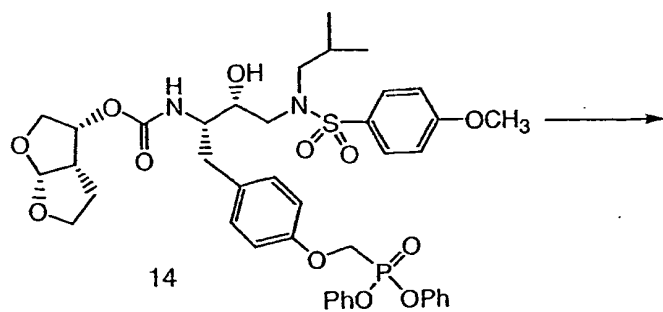
Scheme 5



Scheme 6

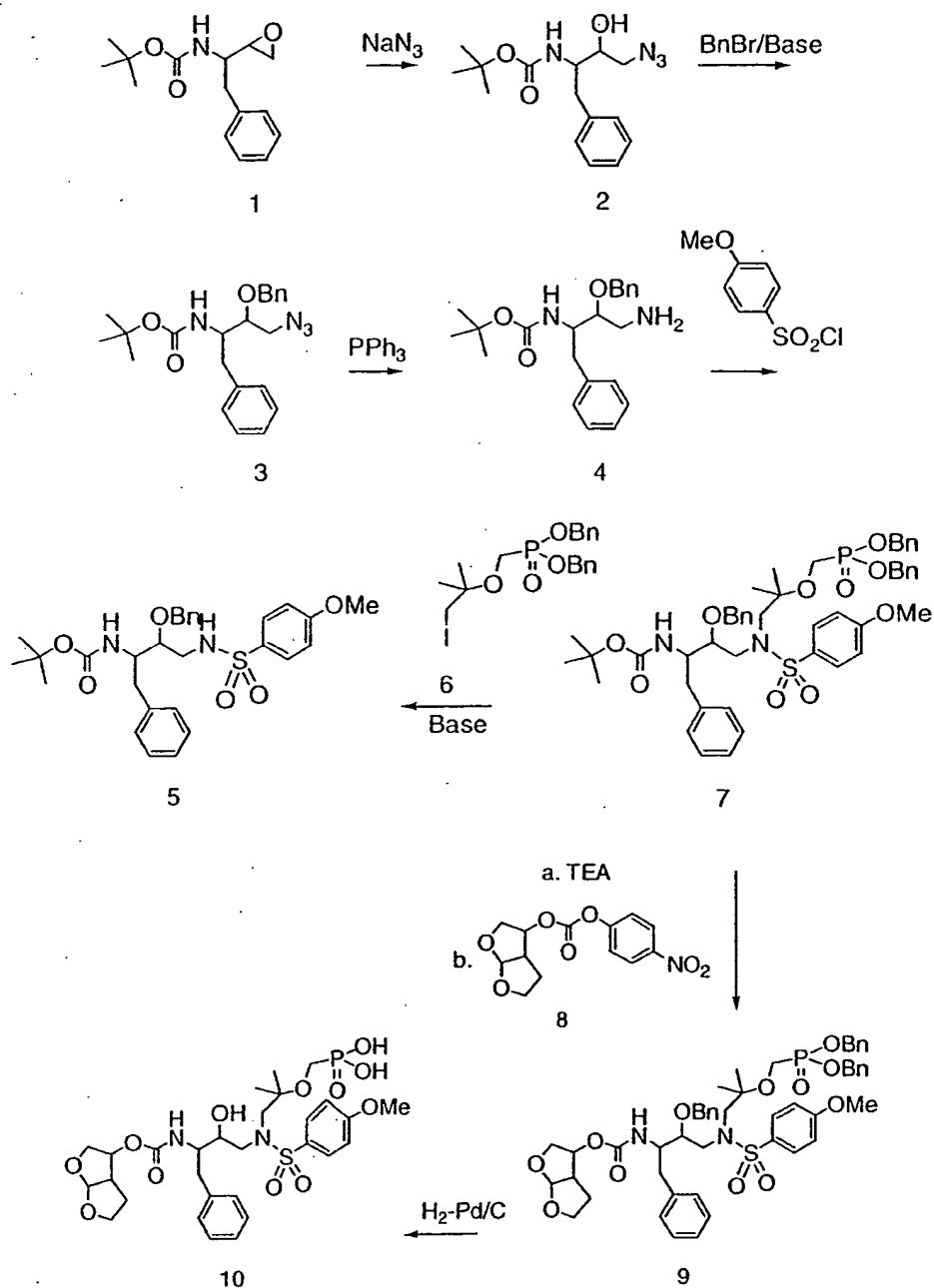


Scheme 7



**Scheme Section B**

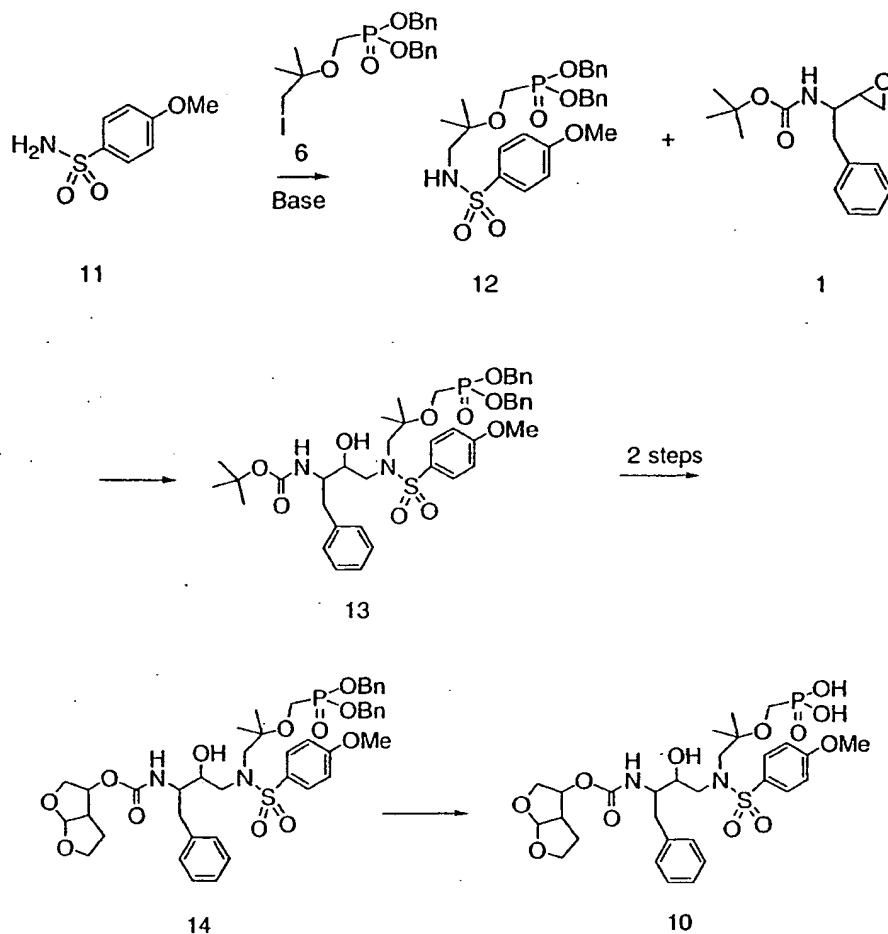
Alternative exemplary methods of preparing the compounds of the invention are shown in Schemes 101-113 below.

**5 Scheme 101**

Treatment of commercially available epoxide 1 with sodium azide (Bioorg. & Med. Chem. Lett., 5, 459, 1995) furnishes the azide intermediate 2. The free hydroxyl is converted to benzyl ether 3 by treating it with benzyl bromide in the presence of base such as potassium carbonate. Compound 4 is achieved by the reduction of the azide group with triphenyl phosphine, as described in the publication Bioorg. & Med. Chem. Lett., 7, 1847, 1997. Conversion of the amino group to its sulfonamide derivative 5 is achieved by treating the amine with stoichiometric amounts of sulfonyl chloride. Regioselective alkylation is performed (as shown in the article J. Med. Chem., 40, 2525, 1997) on the sulfonamide nitrogen using the iodide 6 (J. Med. Chem., 35, 2958, 1992) to get the compound 7. Upon TFA catalyzed deprotection of BOC group followed by the reaction with bisfuranyl carbonate 8 (for a similar coupling see, J. Med. Chem., 39, 3278, 1996) furnishes the compound 9. Final deprotection of the protecting groups by catalytic hydrogenolysis result the compound 10.

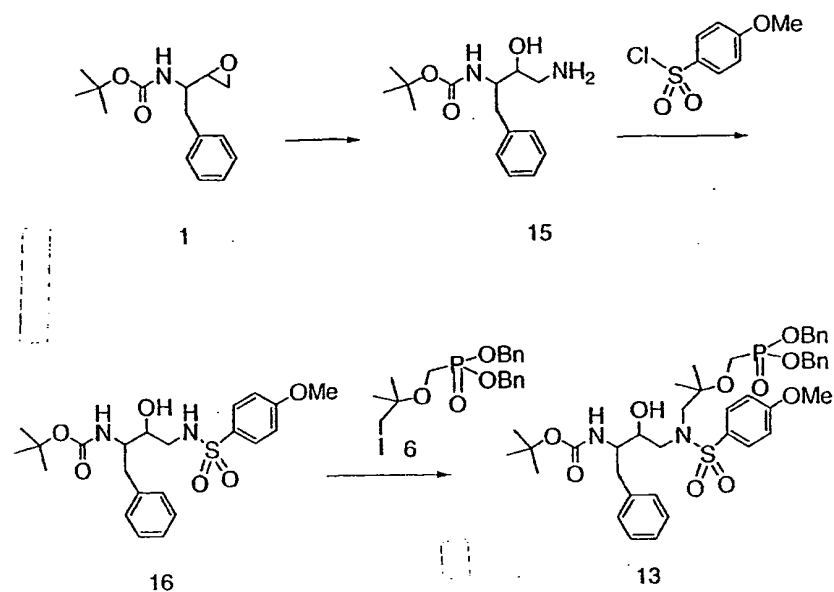


## Scheme 102



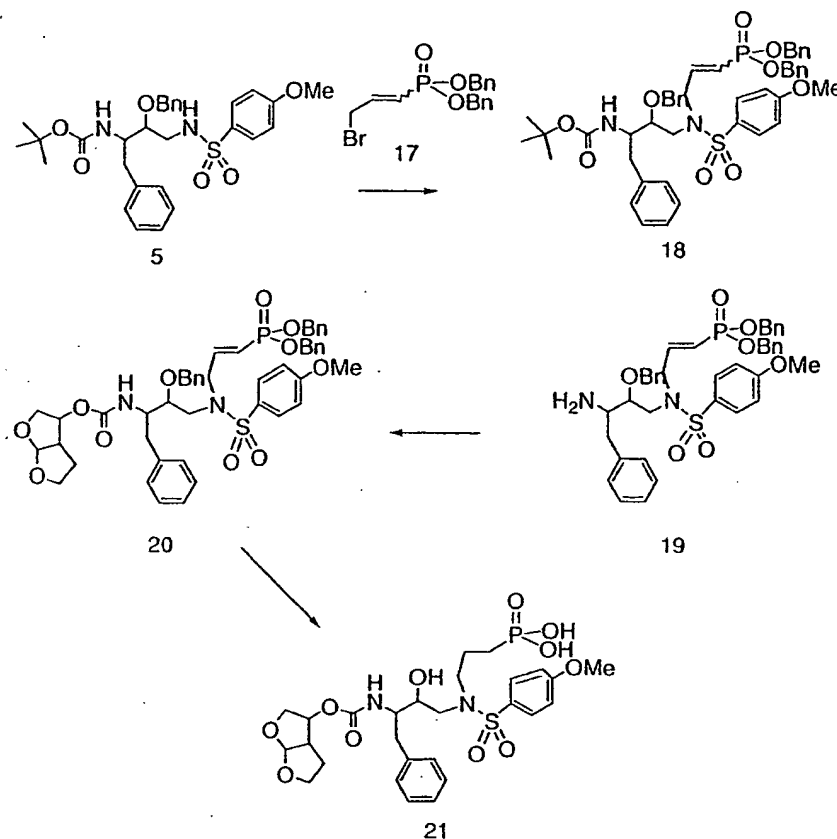
- 10 The sulfonamide 11 is readily alkylated with the iodide 6 (J. Med. Chem., 35, 2958, 1992) to get the intermediate 12. Regioselective epoxide opening (JP -9124630) of the epoxide 1 with 12 furnishes the intermediate 13. Deprotection of the BOC group followed by the treatment of bisfuranyl carbonate 8 yields the intermediate 14 which is subjected to hydrogenation to furnish the compound 10.

Scheme 103



- 5 The epoxide 1 is converted to the aminohydroxyl derivative 15 using the known procedure (J. Med. Chem., 37, 1758, 1994). Sulfonation of 15 using benzene sulfonylchloride affords the compound 16. Installation of the side chain to get the intermediate 13 is achieved by alkylation of sulfonamide nitrogen with iodide 6. The intermediate 13 is converted to the compound 10 using the same sequence as shown in scheme 102.

Scheme 104

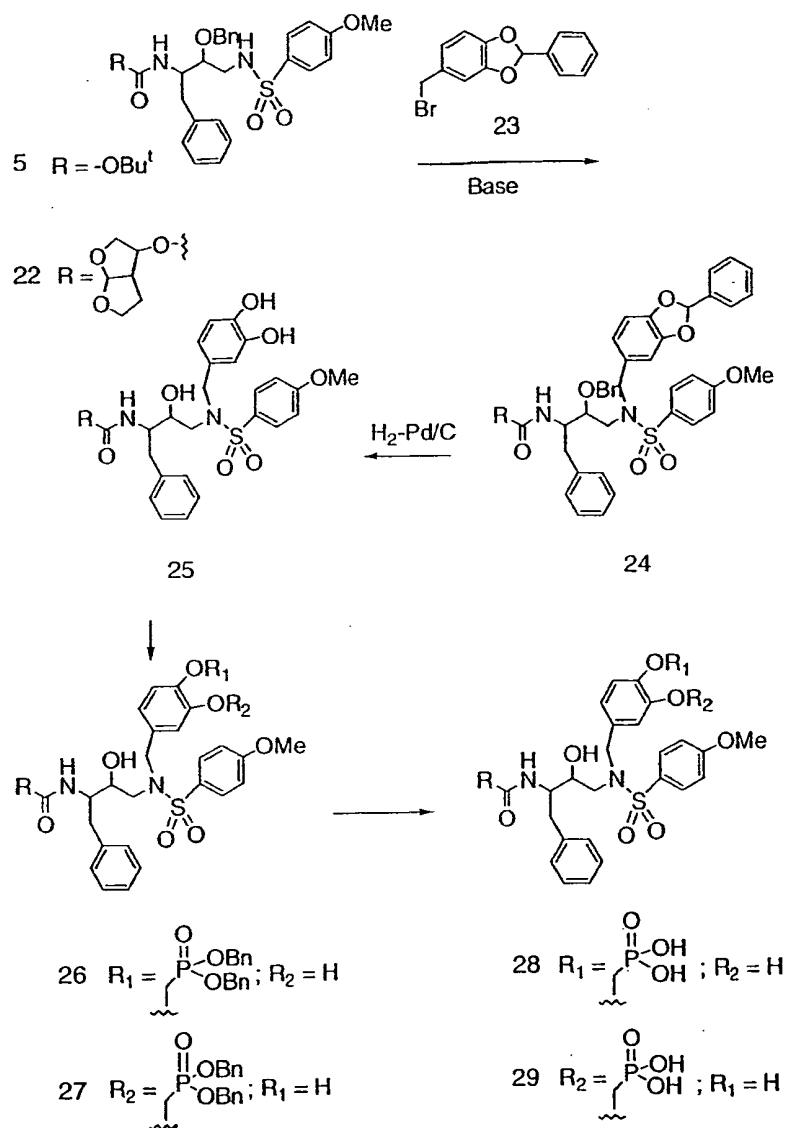


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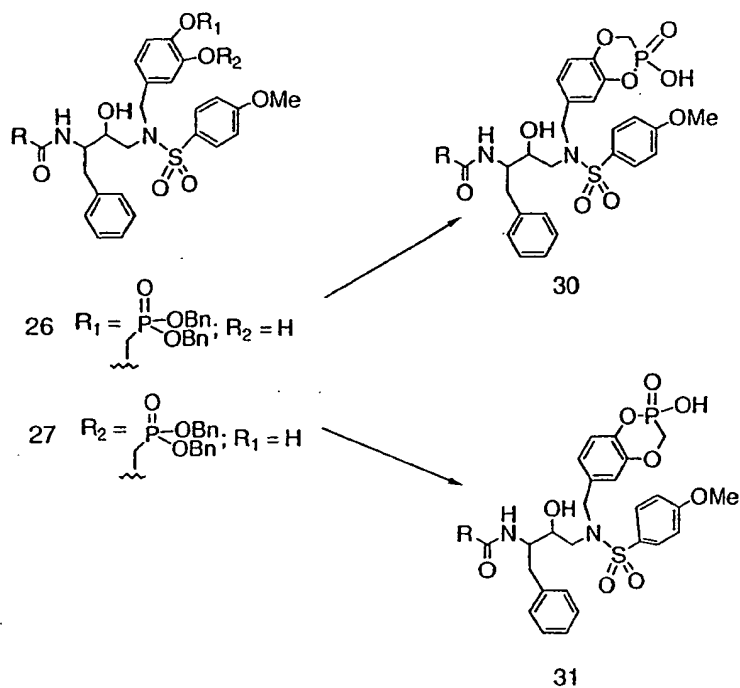
Sulfonamide 5 is alkylated under basic conditions using the allyl bromide 17 (Chem. Pharm. Bull., 30, 111, 1982) to get the intermediate 18. Similar transformation is reported in literature (J. Med. Chem., 40, 2525, 1997). Hydrolysis of BOC group with TFA and acylation of the resulting amine 19 with bisfuranyl carbonate 8 yields the compound 20.

10 Hydrogenation using Pd/C catalysis under H<sub>2</sub> atmosphere affords the phosphonic acid 21.

Scheme 105

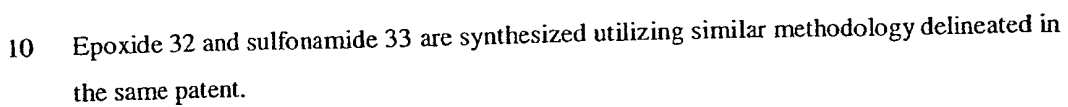


Scheme 105 (cont)

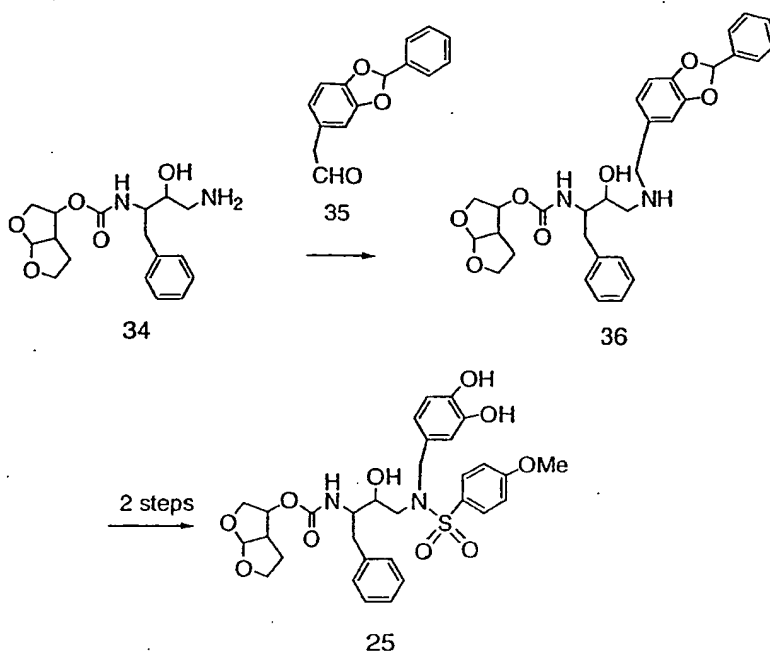


- 5 Sulfonamide 5 is converted to 22 via hydrolysis of BOC group with TFA and acylation with bisfuranyl carbonate 8. The sulfonamide 22 is alkylated with the bromide 23 (J. Med. Chem., 40, 2525, 1997) to get the compound 24, which upon hydrogenolysis gives the catechol 25. Alkylation of the phenolic groups using dibenzylhydroxymethyl phosphonate (J. Org. Chem., 53, 3457, 1988) affords regioisomeric compounds 26 and 27. These compounds 26 and 27
- 10 are hydrogenated to get the phosphonic acids 28 and 29, respectively. Individual cyclic phosphonic acids 30 and 31 are obtained under basic (like NaH) conditions (US 5886179) followed by hydrogenolysis of the dibenzyl ester derivatives 26 and 27.

In this route, compound 25 is obtained by conducting a reaction between the epoxide 32 and the sulfonamide 33 using the conditions described in the Japanese Patent No. 9124630.

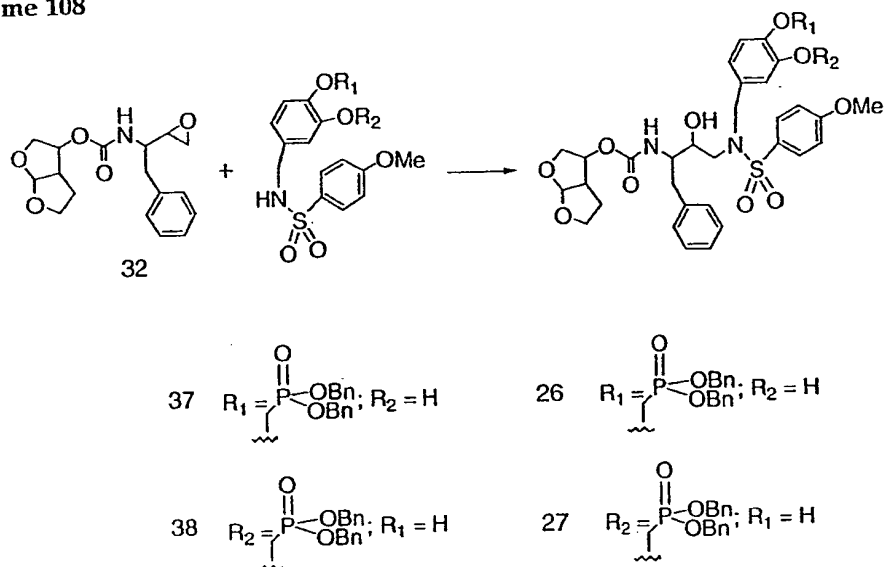


Scheme 107



- 5 Compound 34 is obtained from 32 using similar sequence depicted in J. Med. Chem., 37, 1758, 1994. Reductive amination (for similar transformation see WO 00/47551) of compound 34 with aldehyde 35 furnishes the intermediate 36 which is converted to the compound 25 by sulfonation followed by hydrogenation.

Scheme 108

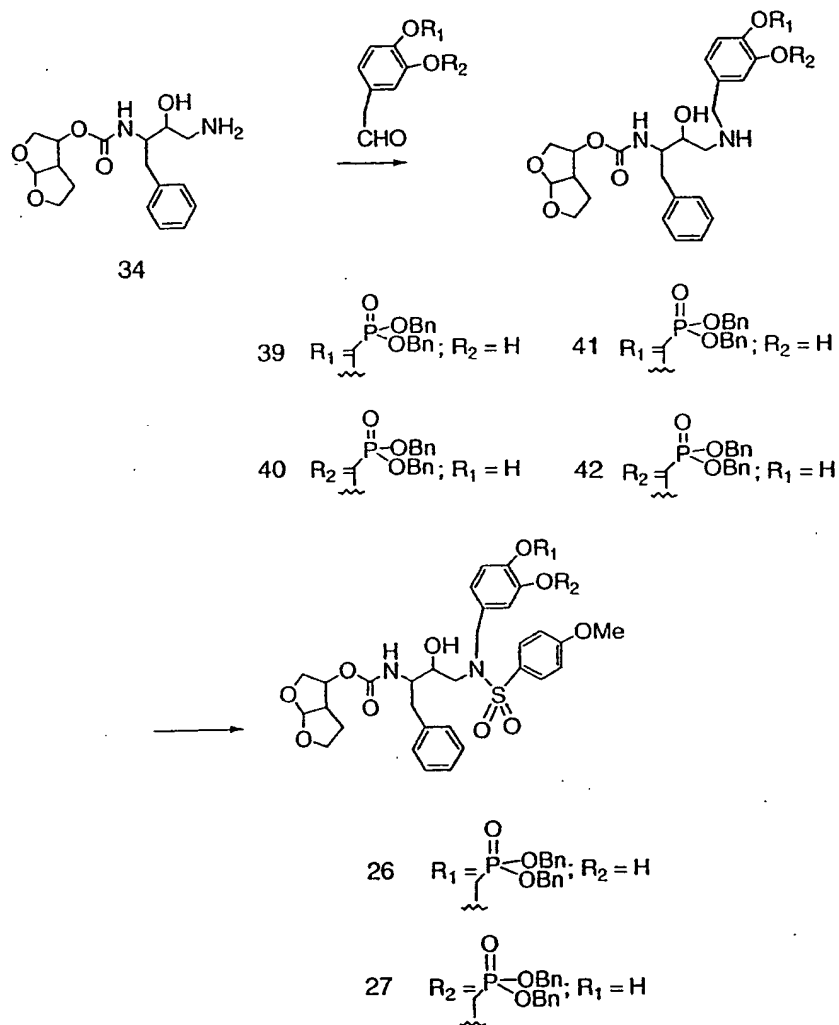


- 5 Treatment of epoxide 32 with sulfonamides 37 and/or 38 under conditions described in Japanese Patent No. 9124630 furnishes 26 and 27.



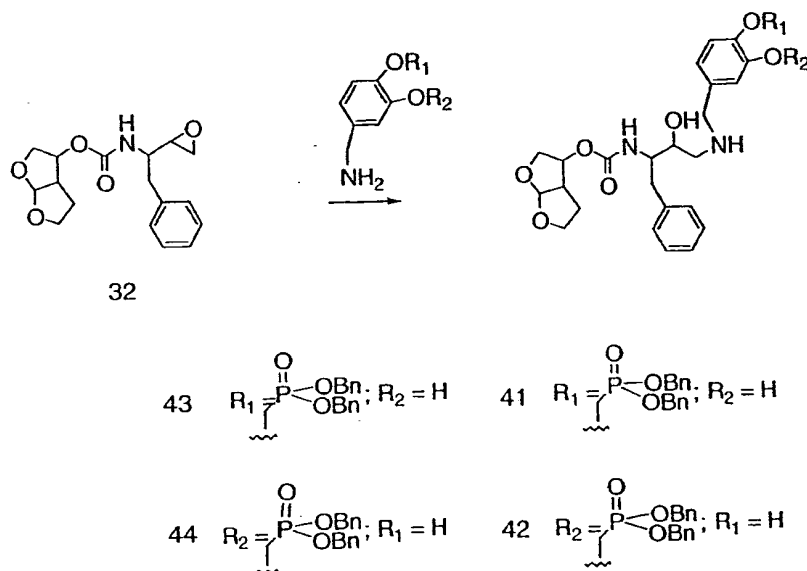
## Scheme 109

Reductive amination of aminohydroxyl intermediate 34 with the aldehydes 39 and 40 as described in patent WO 00/47551, furnish 41 and 42 which undergoes smooth sulfonylation to give 26 and 27.

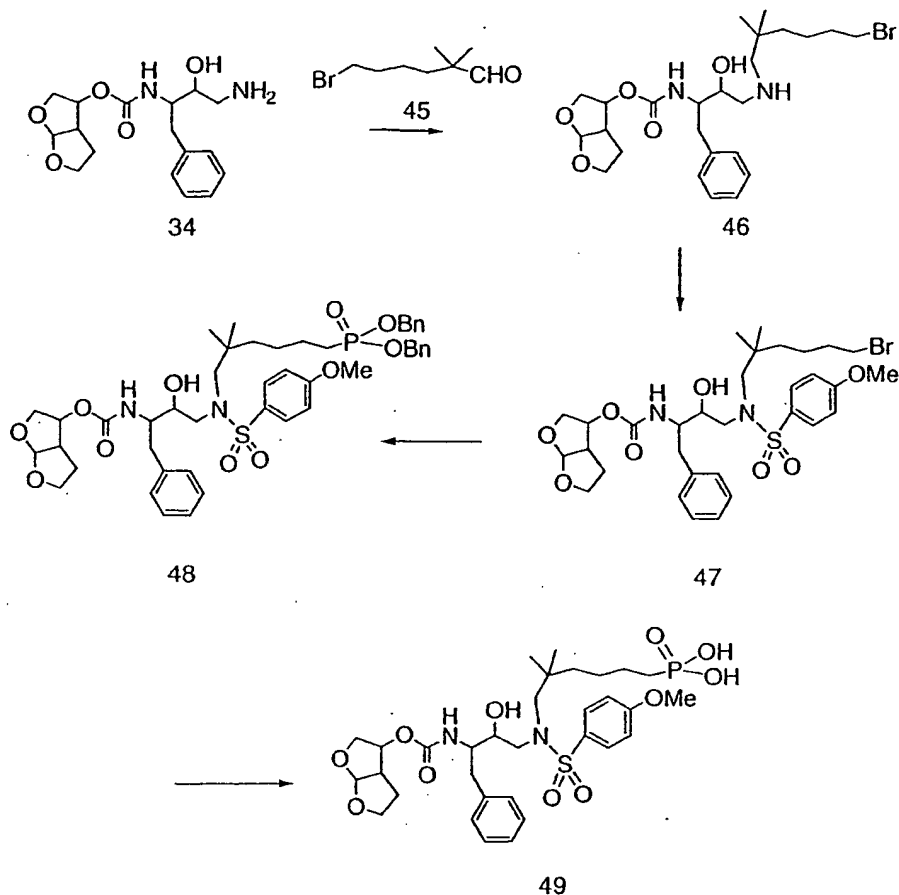


## Scheme 110

In an alternate approach, where epoxide 32 is opened with benzyl amines 43 and 44 under conditions described above furnishes 41 and 42, respectively. Similar transformations were documented in the Japanese Patent No. 9124630.



Scheme 111

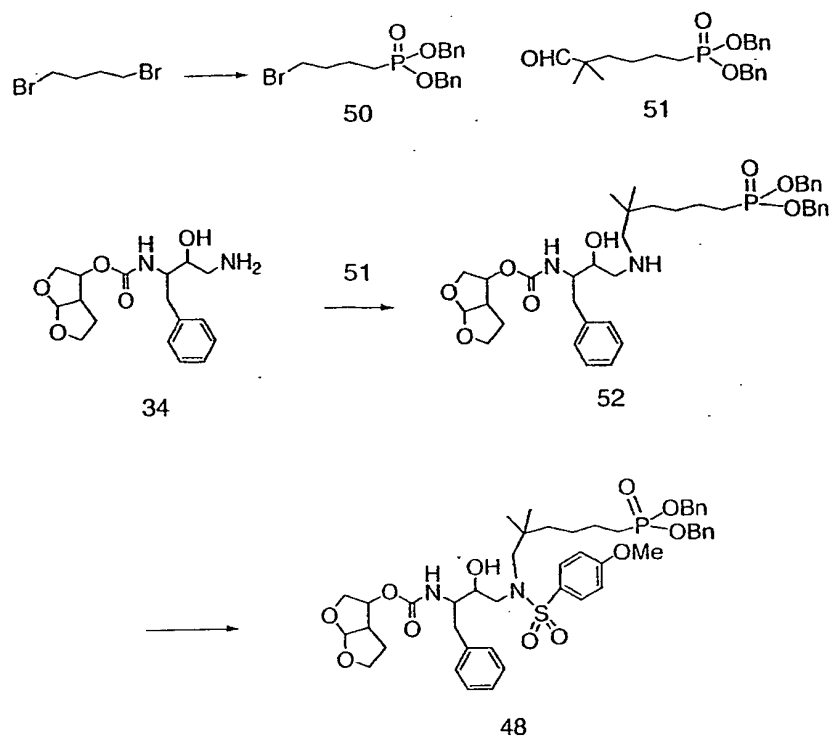


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Reductive amination of the bromoaldehyde 45 (J. Organomet. Chem., FR; 122, 123, 1976) with the amine 34 gives 46 which then undergoes sulfonylation to furnish 47. The bromoderivative 47 is converted to the phosphonate 48 under Michaelis-Arbuzov reaction conditions (Bioorg. Med. Chem. Lett., 9, 3069, 1999). Final hydrogenation of 48 delivers the phosphonic acid 49.

10

## Scheme 112

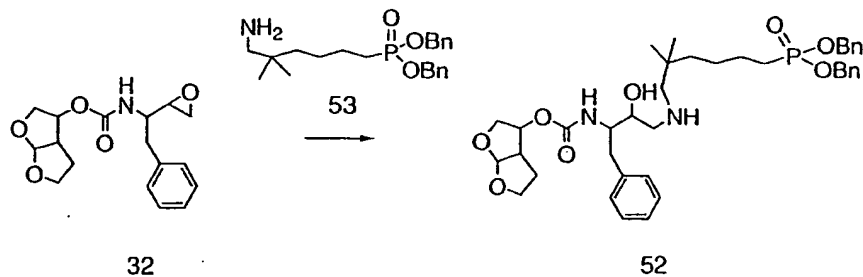


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The intermediate 48 is also obtained as shown in scheme 112. Reductive amination of the aldehyde 52 with the amine 34 offers the phosphonate 52 and sulfonylation of this intermediate furnishes 48.

## Scheme 113

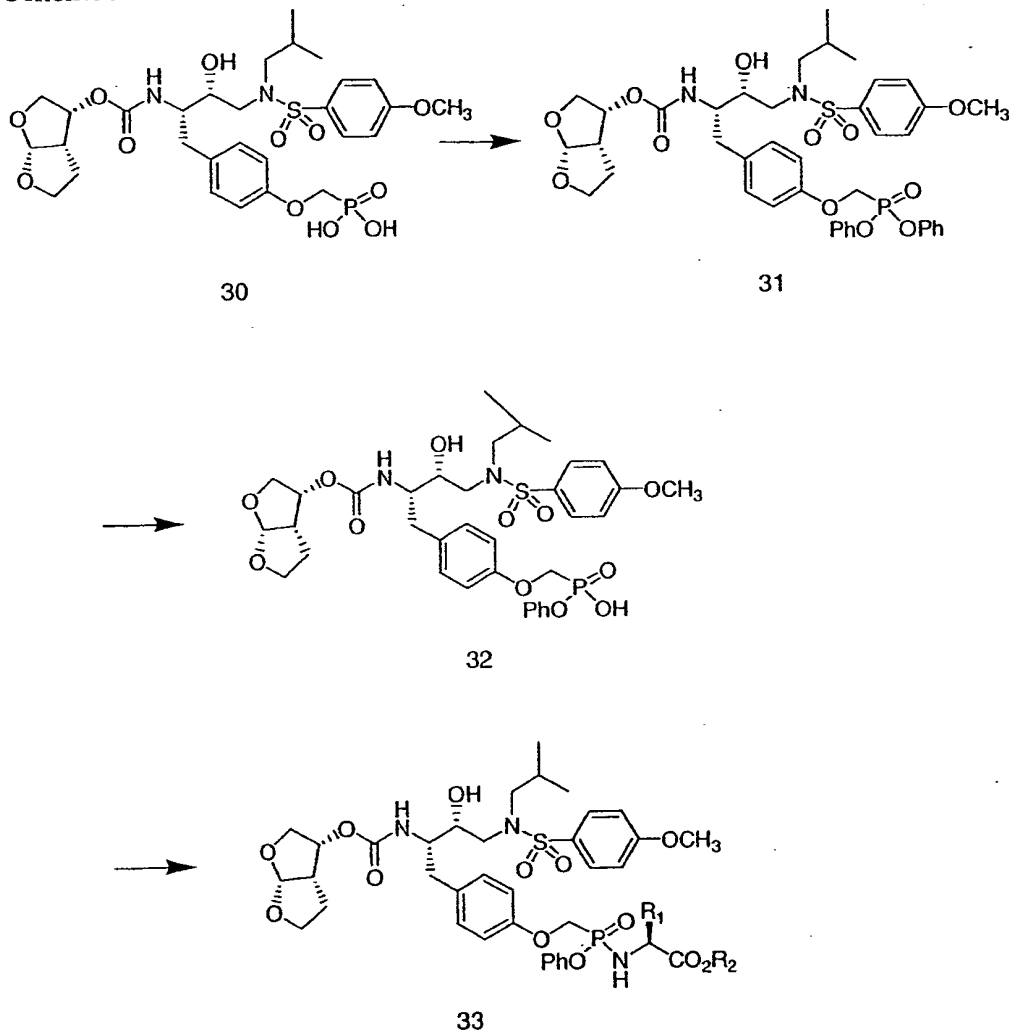


10 Alternatively, compound 52 is obtained from the epoxide 32 by a ring opening reaction with the aminophosphonate 53 (Scheme 113).

Scheme Section C

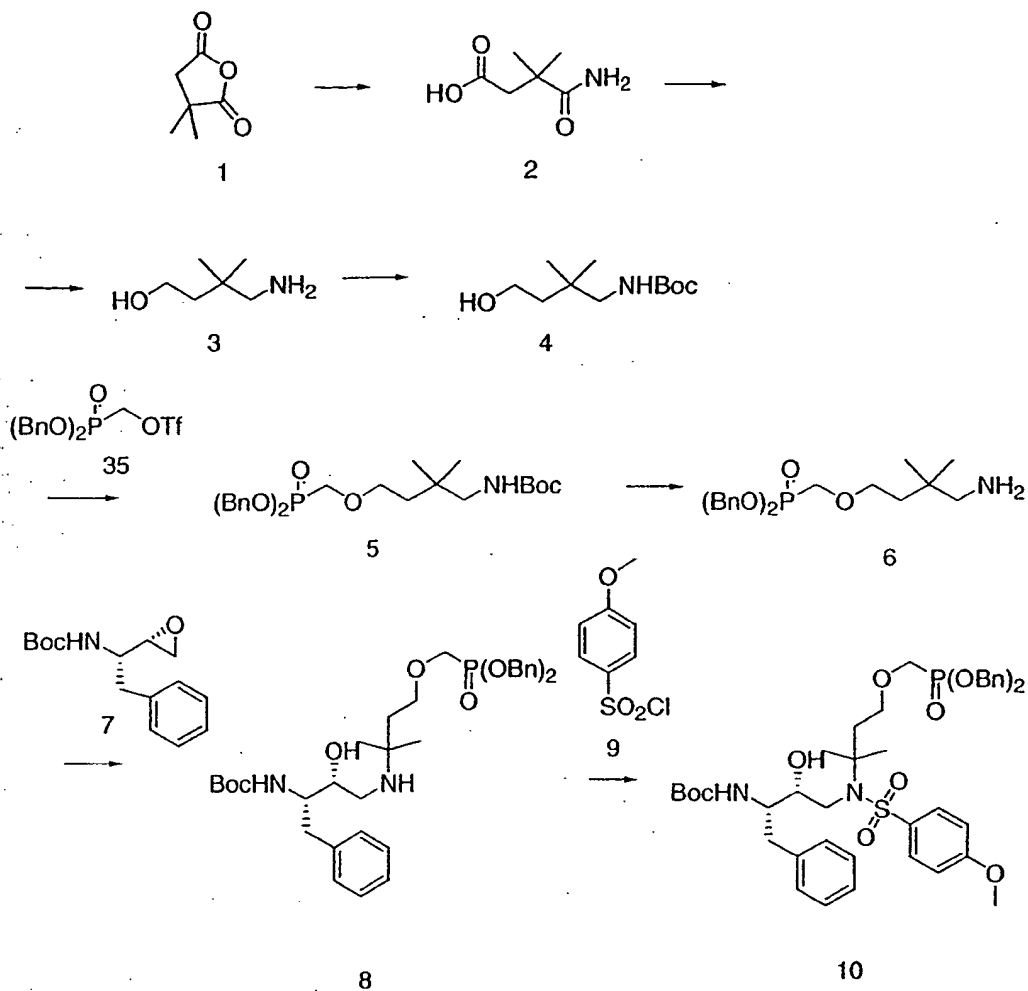
Scheme 9 is described in the Examples.

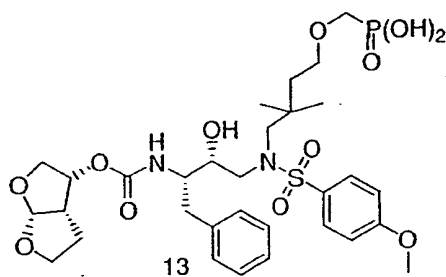
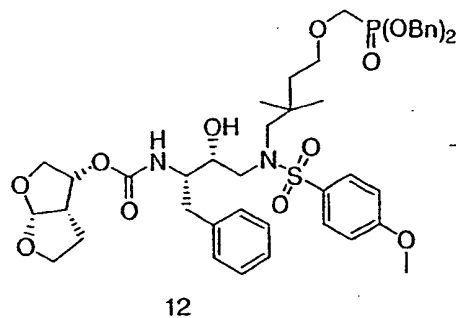
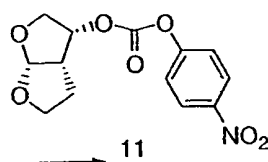
## 5 Scheme 9



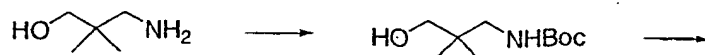
**Scheme Section D**

The following schemes are described in the Examples.









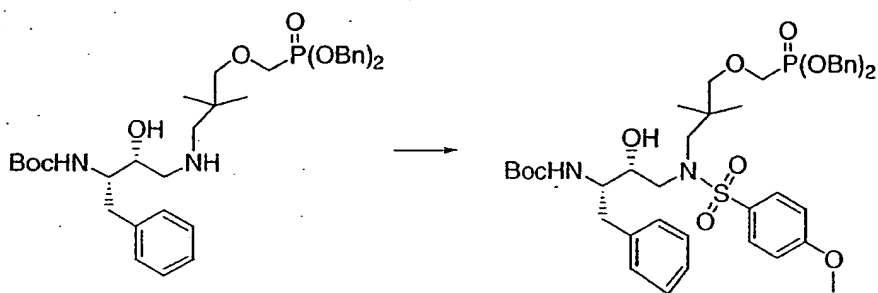
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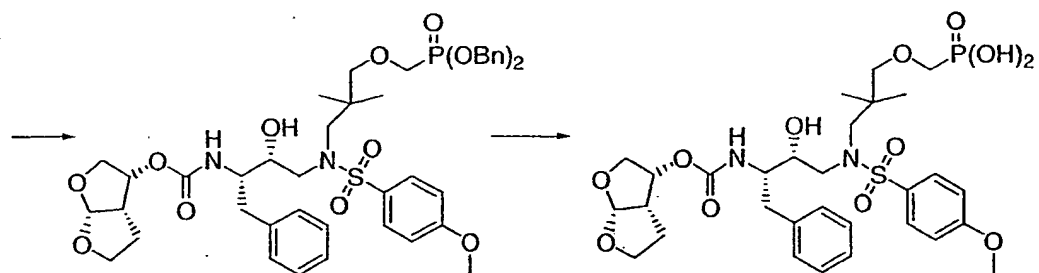
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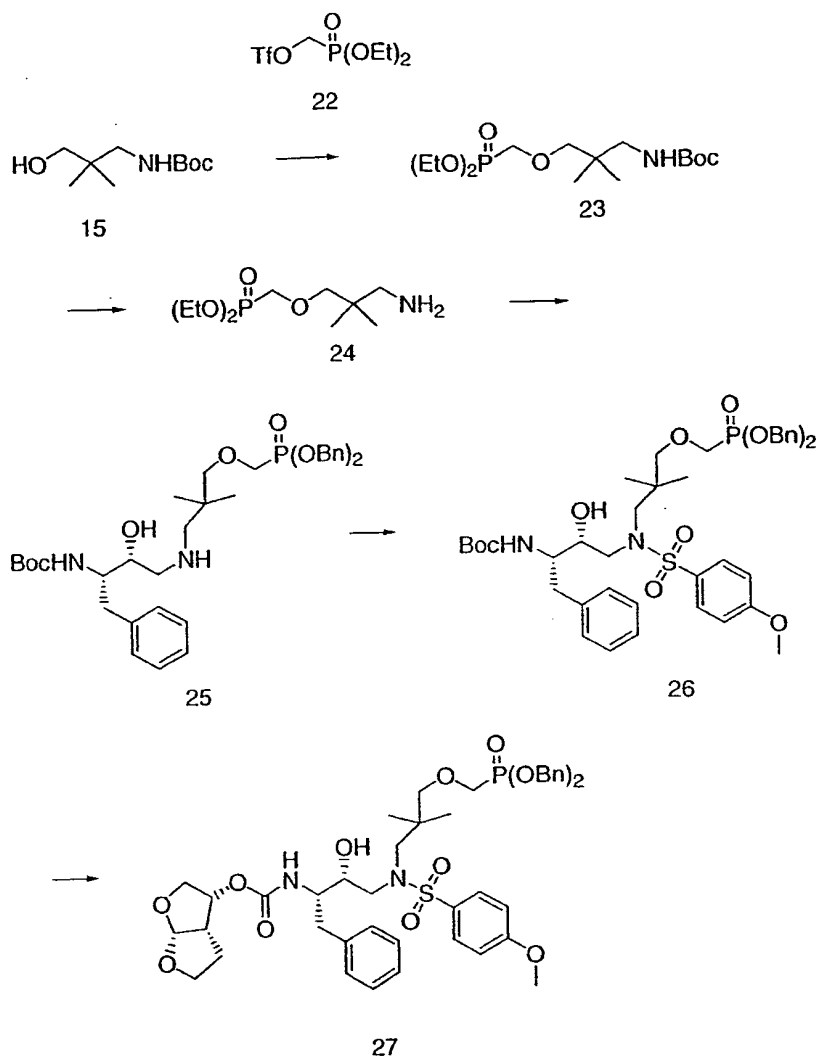
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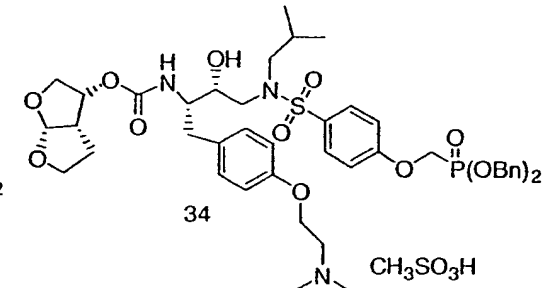
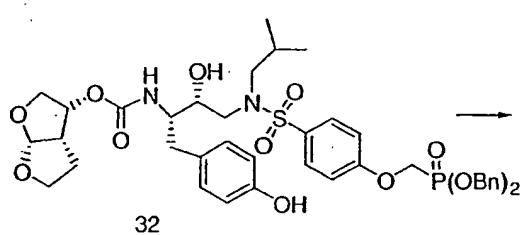
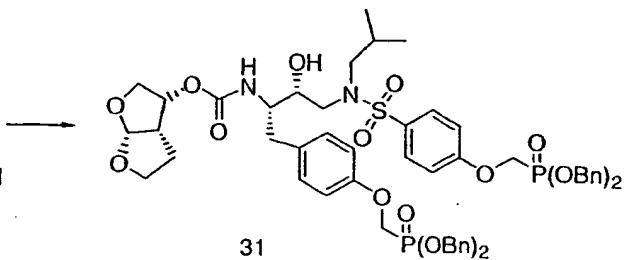
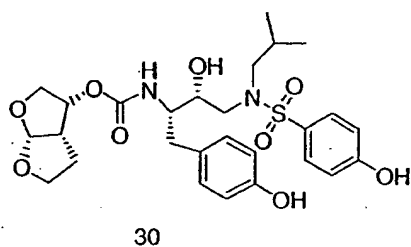
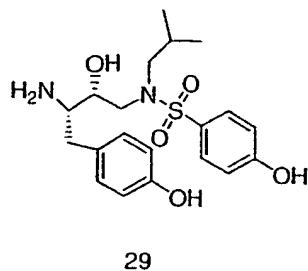
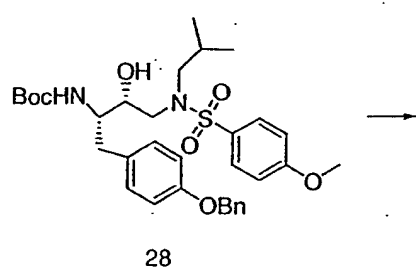
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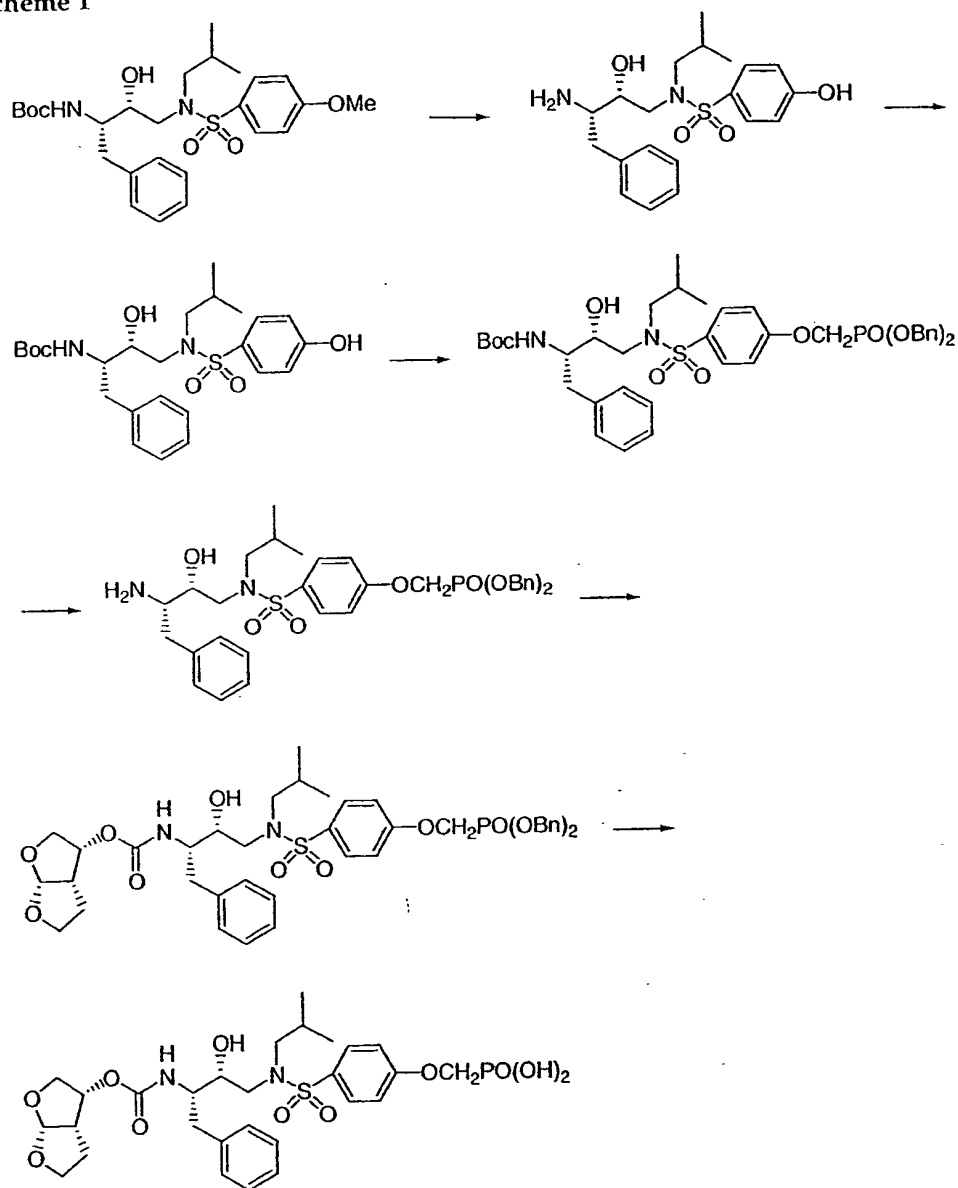
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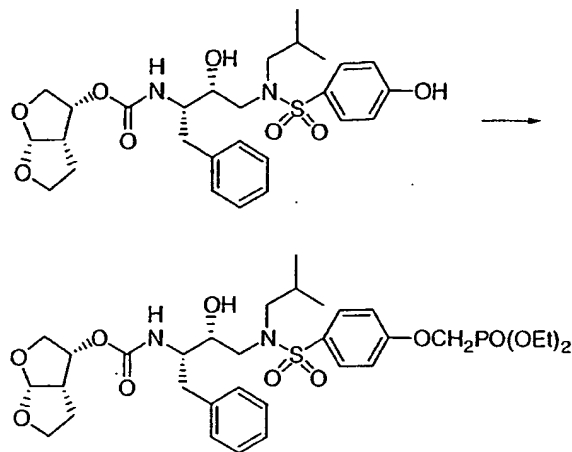


**Scheme Section E**

Schemes 1-3 are described in the examples.

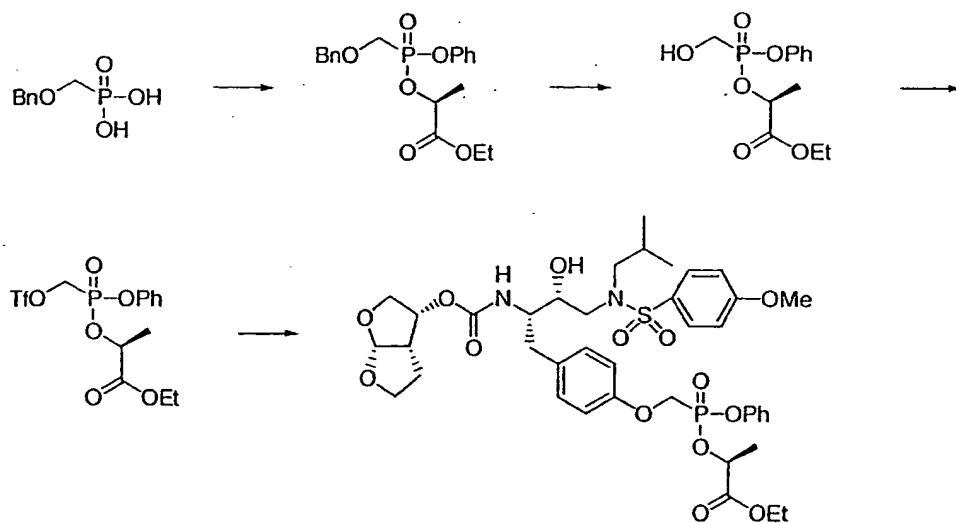
**Scheme 1**

Scheme 2



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Scheme 3

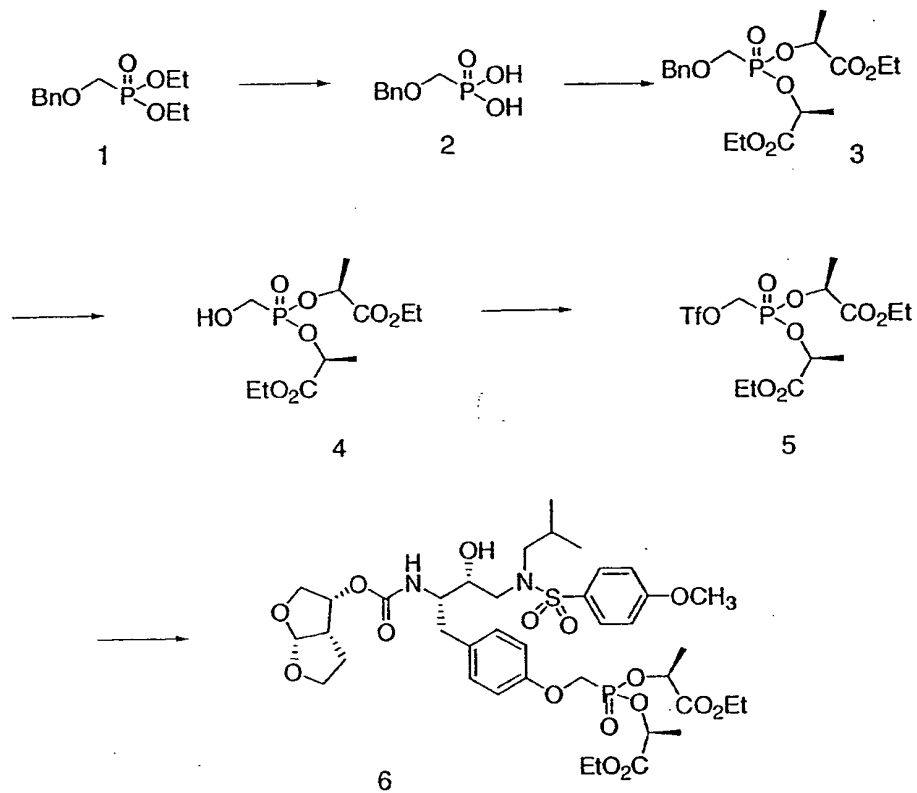


**Scheme Section F**

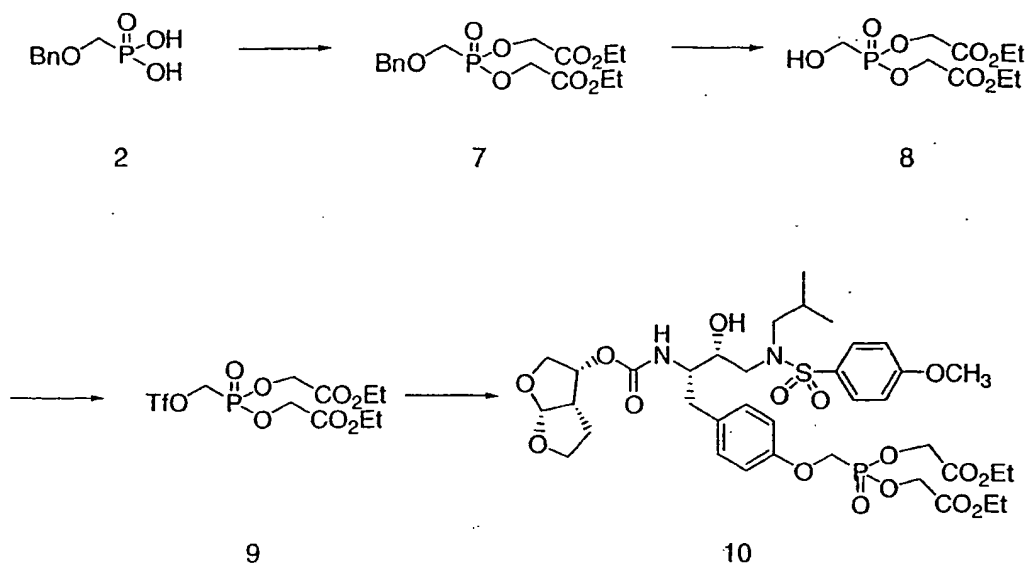
Schemes 1-5 are described in the examples.

**Scheme 1**

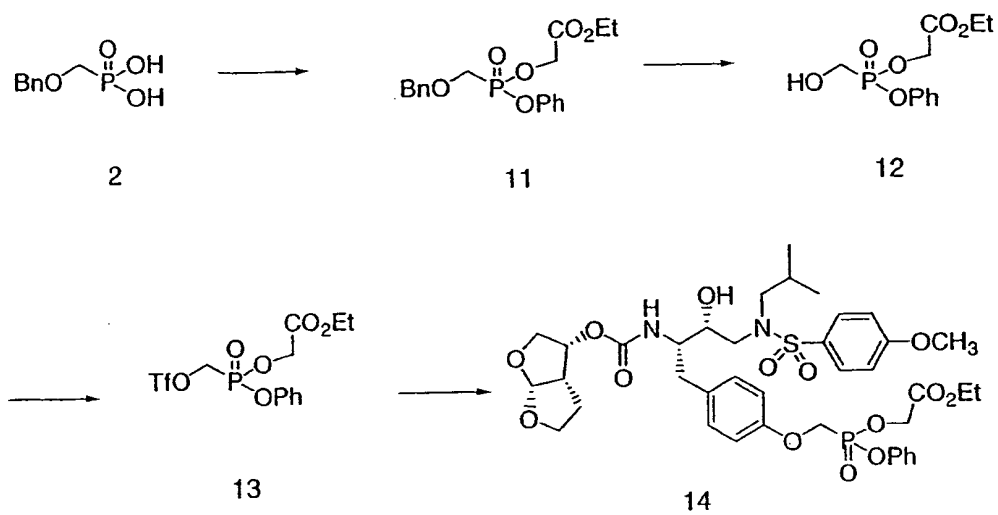
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Scheme 2

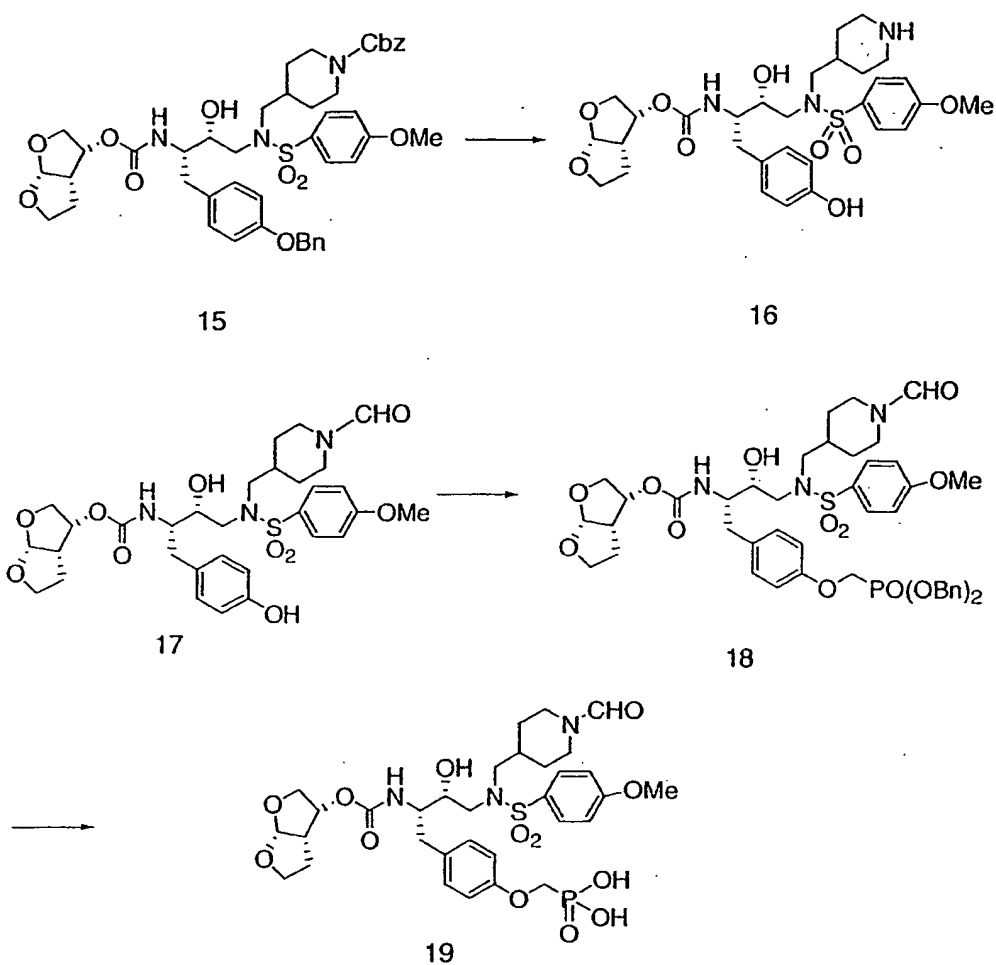


## Scheme 3

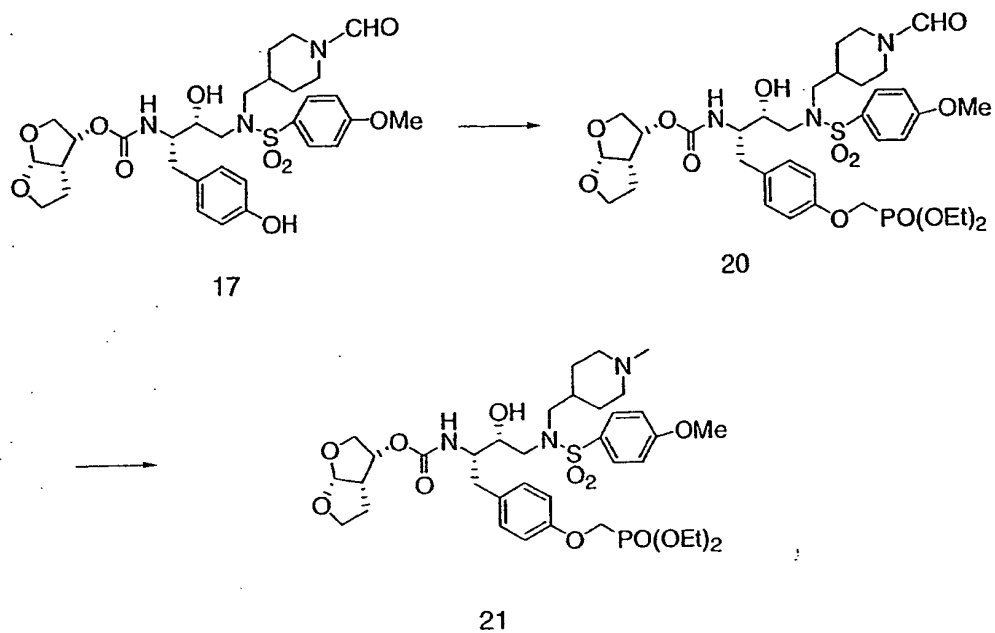




Scheme 4

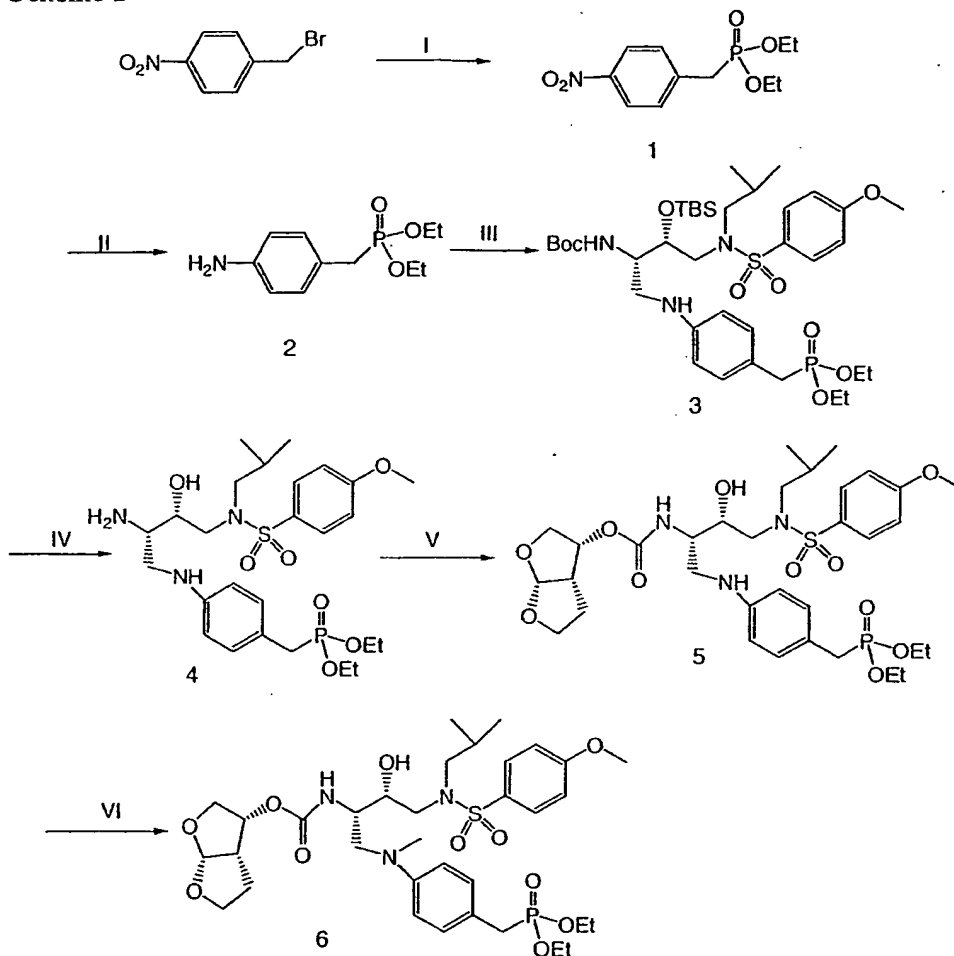


Scheme 5



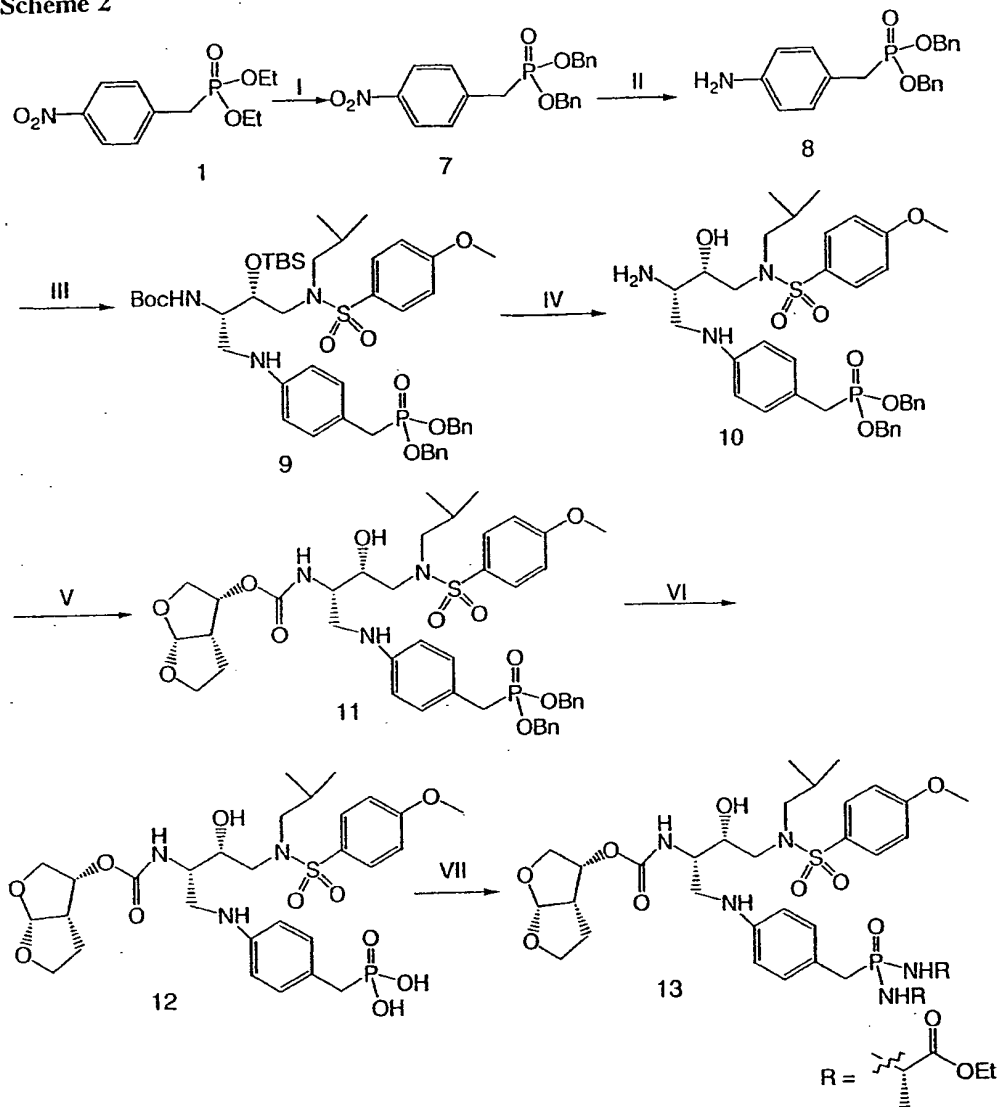
**Scheme Section G**

Schemes 1 to 9 are described in the examples.

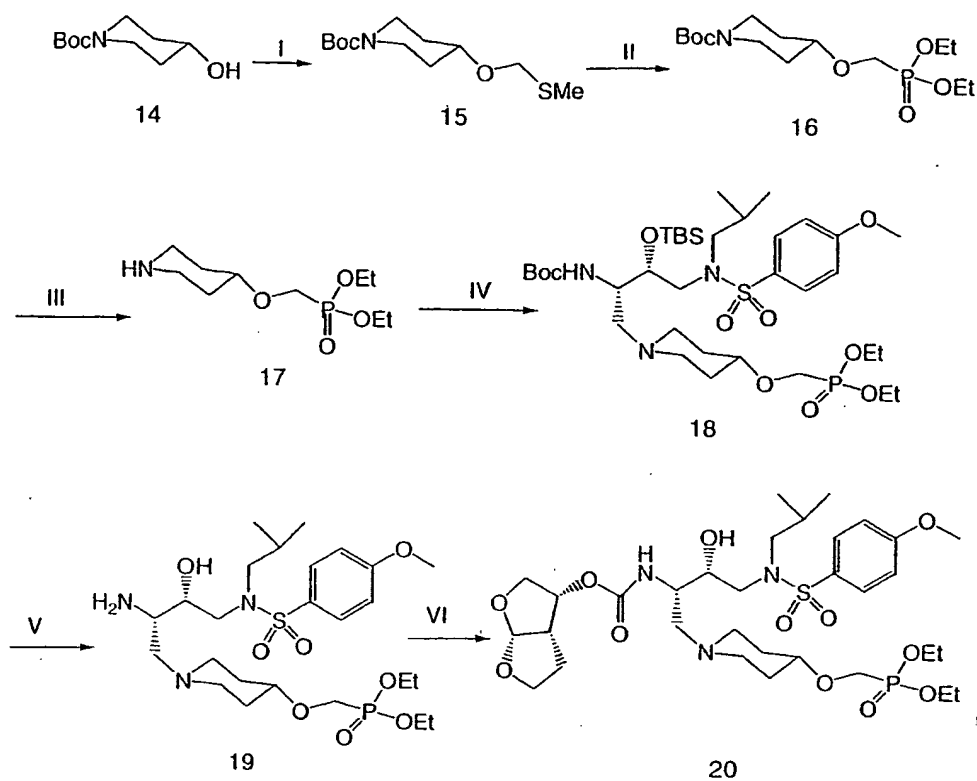
**Scheme 1**

I.  $P(OEt)_3/120\text{ }^\circ\text{C}$ ; II.  $H_2/10\%\text{Pd-C}$ ; III. See Scheme Section H, Scheme 13, Compound 48 /  $NaBH_3CN/HOAc/MeOH$ ; IV. a. TFA; b.  $n-Bu_4NF$ ; V. bisfurancarboxylic anhydride/DMAP; VI.  $HCHO/NaBH_3CN/HOAc/MeOH$

Scheme 2

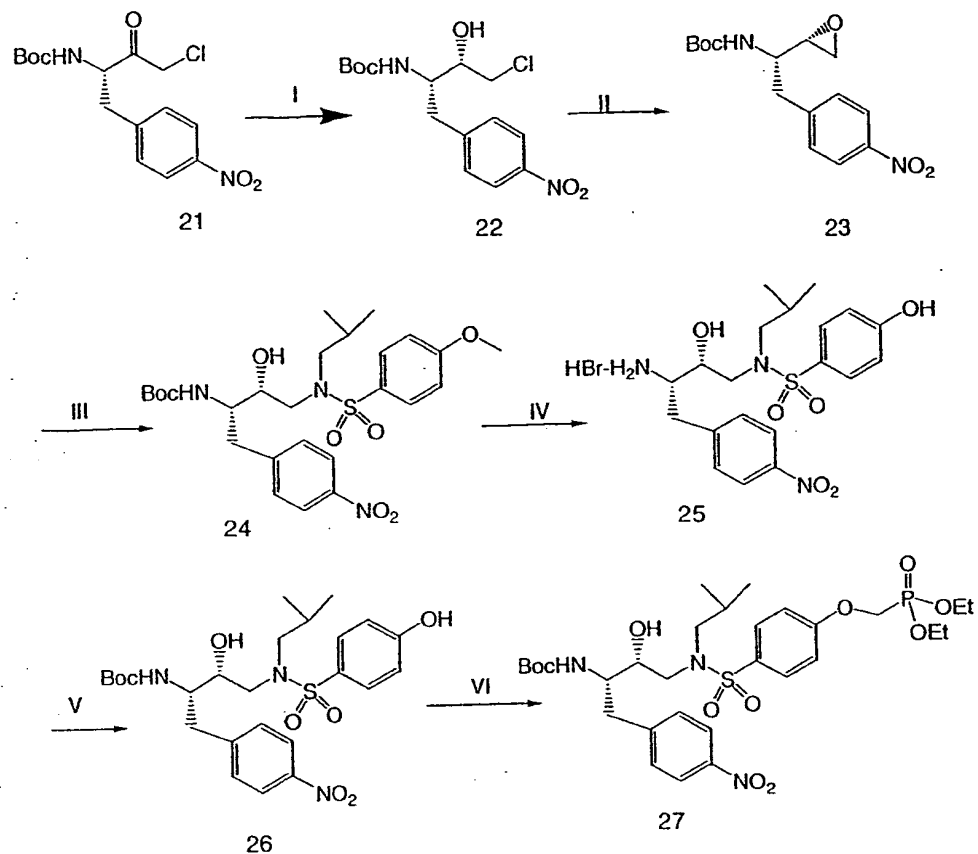


Scheme 3



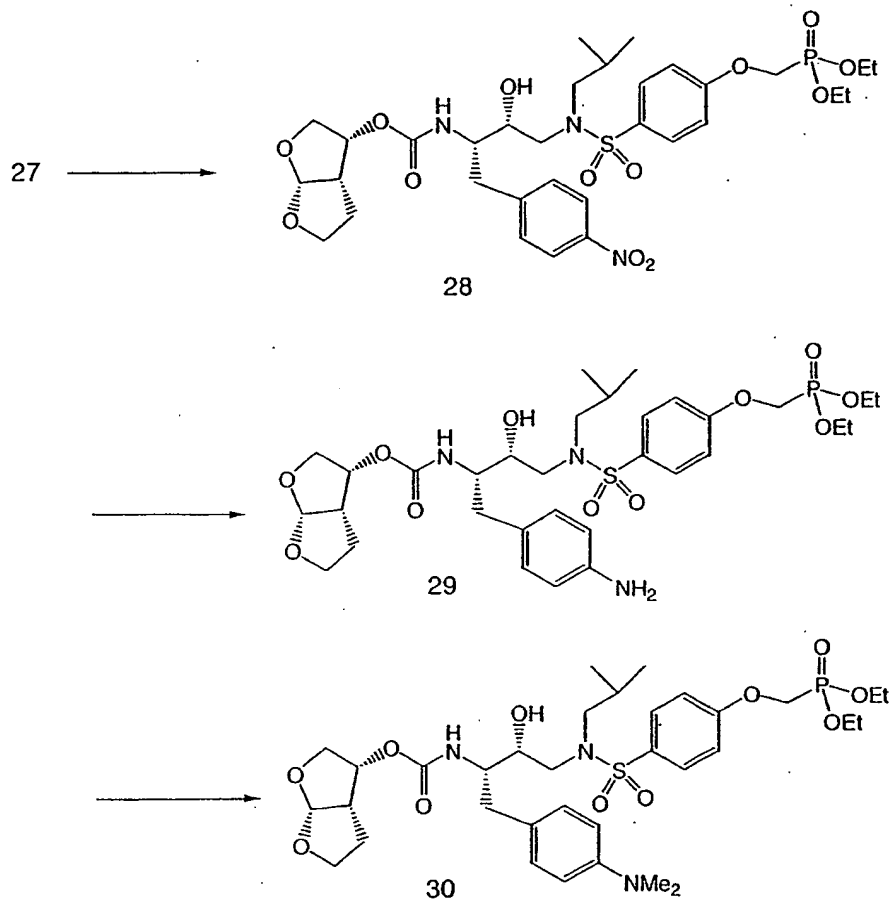
I. a. NaH; b. MTMCl; II. a. SOCl<sub>2</sub>; b. P(OEt)<sub>3</sub>/120 °C; III. TFA; IV. See Scheme Section H, Scheme 13, Compound 48 / NaBH<sub>3</sub>CN/HOAc/MeOH; V. a. TFA; b. n-Bu<sub>4</sub>NF; VI. bisfurancarboxylate/DMAP

Scheme 4



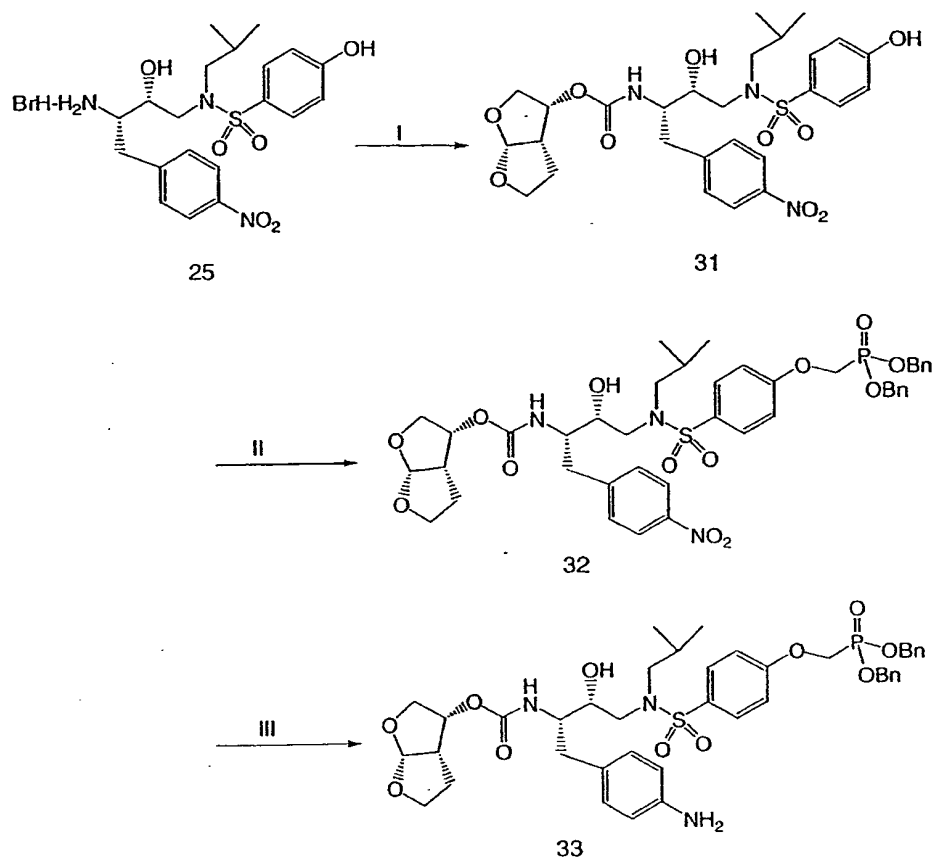
I.  $\text{NaBH}_4/\text{THF}/\text{H}_2\text{O}$ ; II.  $\text{KOH}/\text{EtOH}$ ; III. a. isobutylamine/isopropanol/80 °C; b. 4-methoxybenzenesulfonyl chloride/ $\text{Et}_3\text{N}$ ; IV.  $\text{BBr}_3/\text{CH}_2\text{Cl}_2$ ; V.  $\text{Boc}_2\text{O}/\text{NaHCO}_3$ ; VI.  $\text{Ti}(\text{OCH}_2\text{PO}(\text{OEt})_2)_2/\text{Cs}_2\text{CO}_3$

## Scheme 5



I. TFA/CH<sub>2</sub>Cl<sub>2</sub>; b. bisfurancarboxylate/DMAPI ; II. H<sub>2</sub>/10% Pd-C/EtOH;  
III. HCHO/NaBH<sub>3</sub>CN/HOAc/MeOH

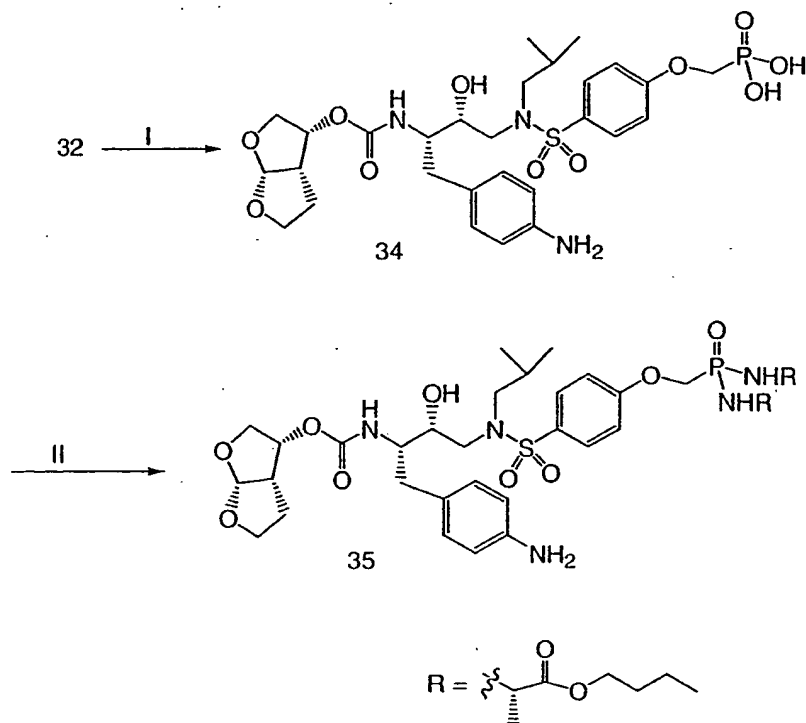
Scheme 6



I. a.  $\text{TMSCl}/\text{Et}_3\text{N}$ ; b. bisfurancarboxylate/ $\text{DMAP}$ ; c.  $\text{n-Bu}_4\text{NF}/\text{HOAc}$ ; II.  $\text{Ti}(\text{OCH}_2\text{PO}(\text{OBn})_2)_2/\text{Cs}_2\text{CO}_3$ ; III.  $\text{Zn}/\text{HOAc}$

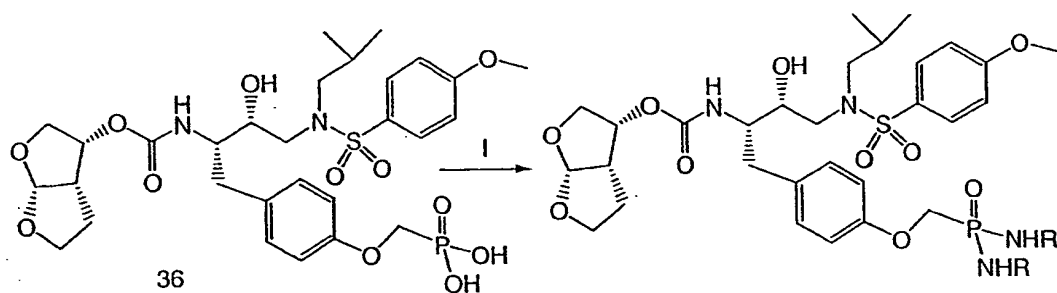


Scheme 7

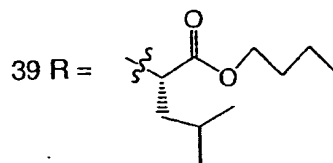
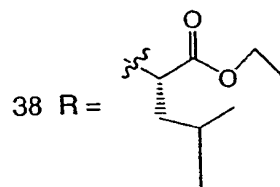
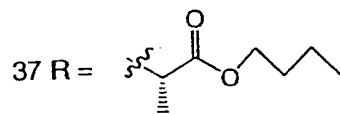


I.  $\text{H}_2/10\% \text{ Pd-C}$ ; II.  $\text{RNH}_2/\text{PPh}_3/\text{Aldrithiol}/\text{diisopropylethylamine}/\text{pyridine}$

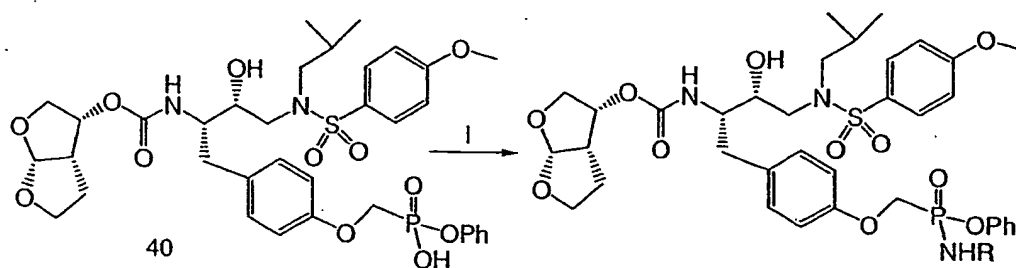
Scheme 8



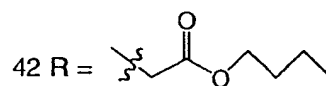
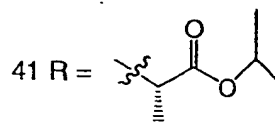
I.  $\text{RNH}_2/\text{PPh}_3/\text{Aldrithiol}/\text{diisopropylethylamine}/\text{pyridine}$



Scheme 9

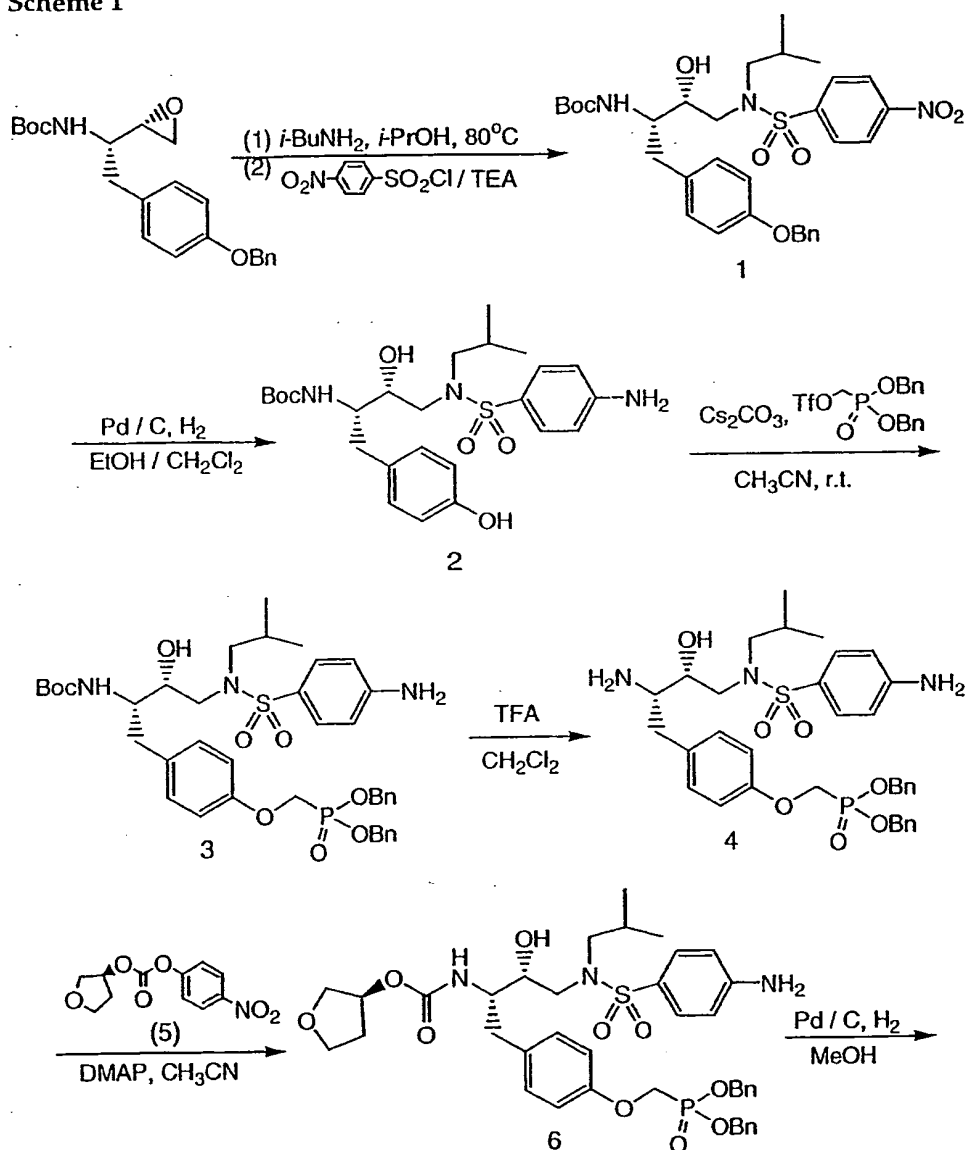


I.  $\text{RNH}_2/\text{PPh}_3/\text{Aldrithiol}/\text{diisopropylethylamine}/\text{pyridine}$

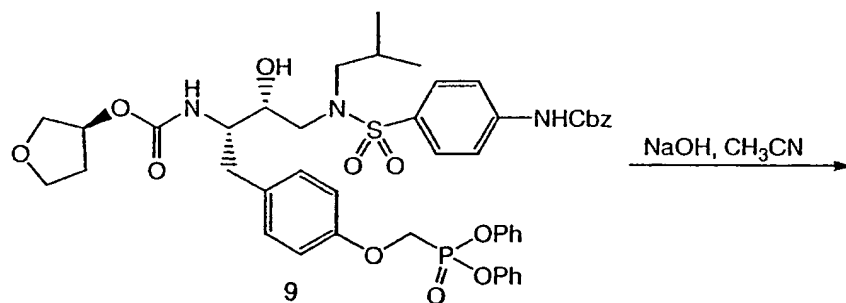
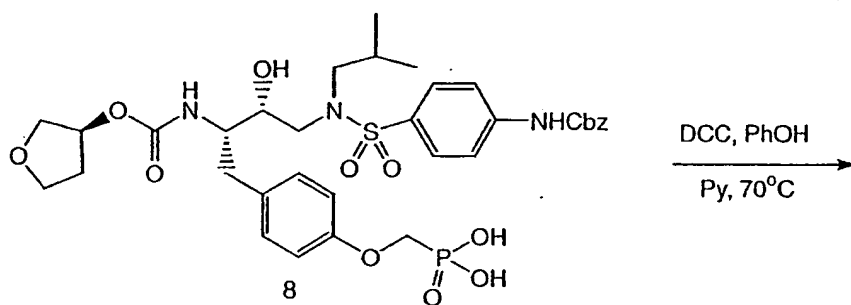
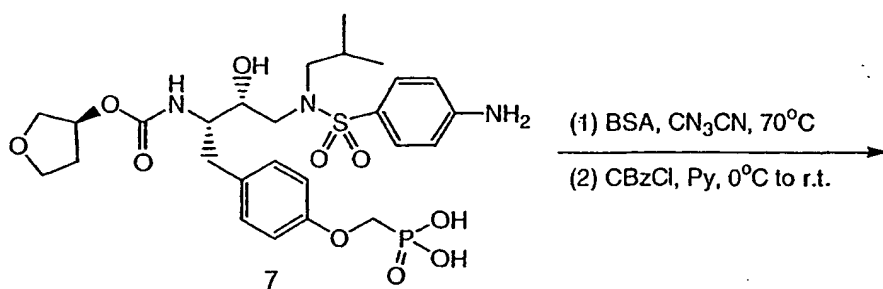


**Scheme Section H**

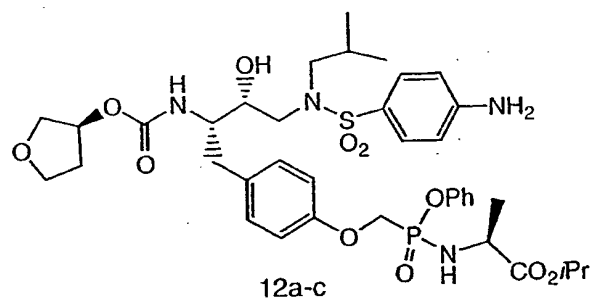
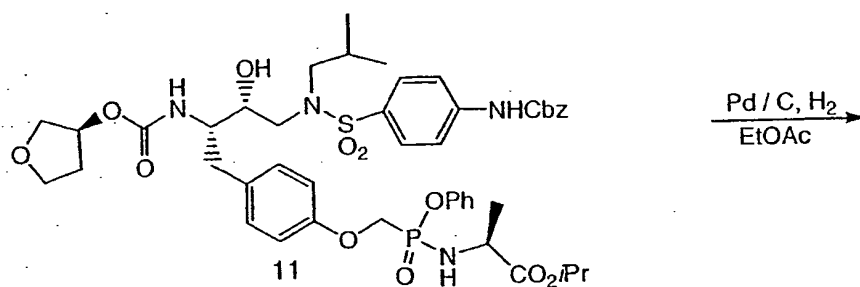
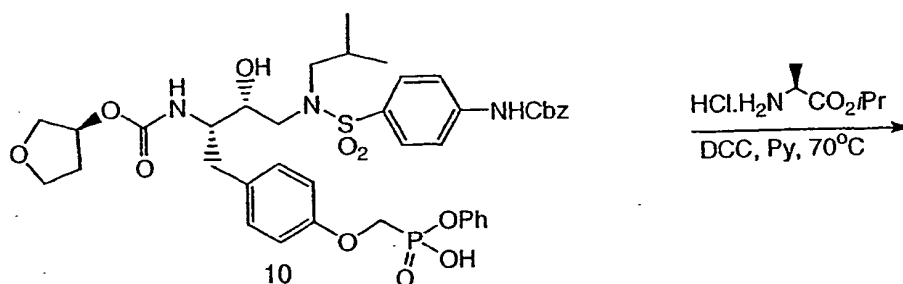
Schemes 1-14 are described in the examples.

**Scheme 1**

Scheme 2

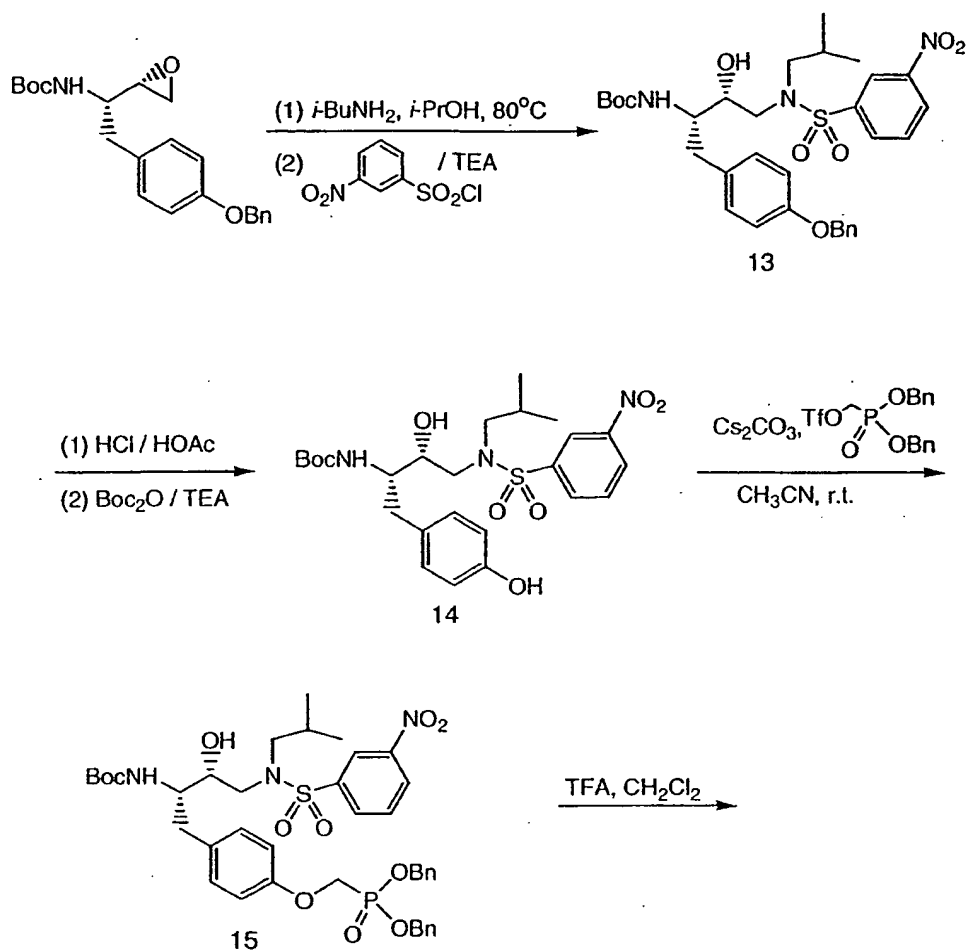


Scheme 3

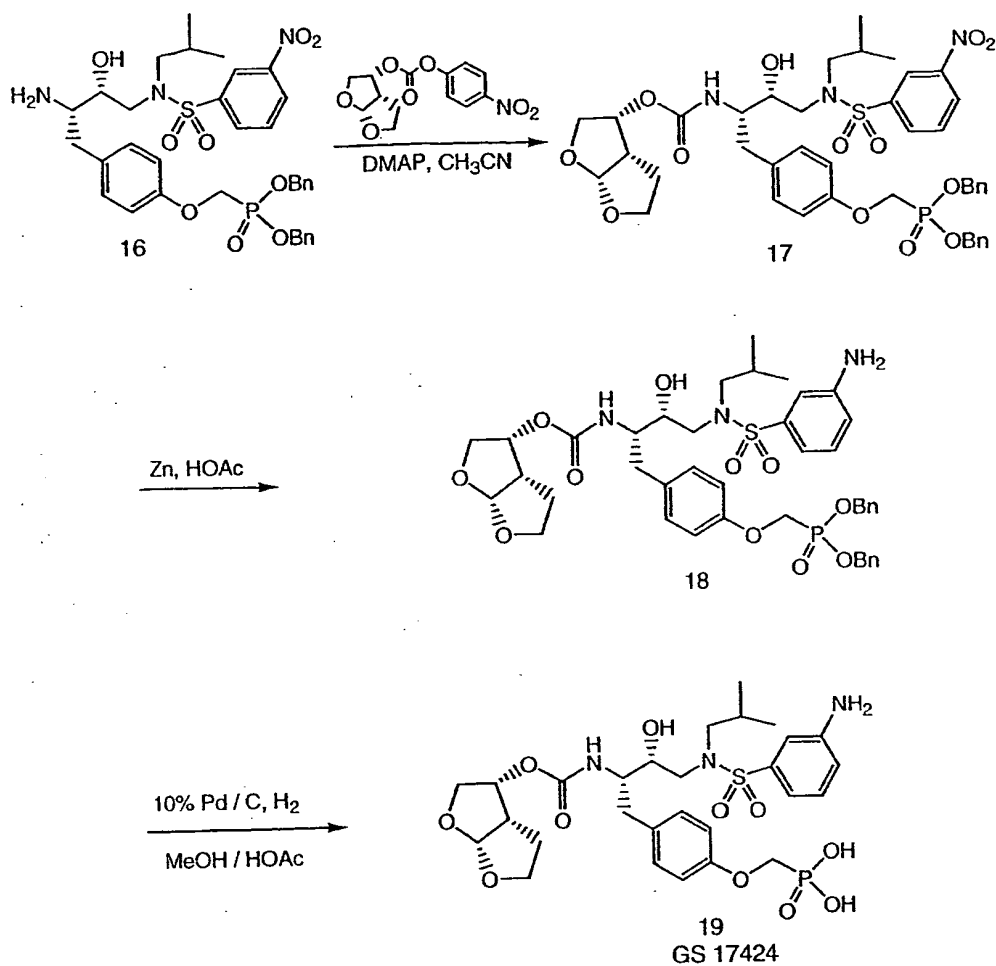


12a, GS 108577 (isomer A / B = 1 : 1)  
12b, GS 108578 (isomer A)  
12c, GS 108579 (isomer B)

Scheme 4

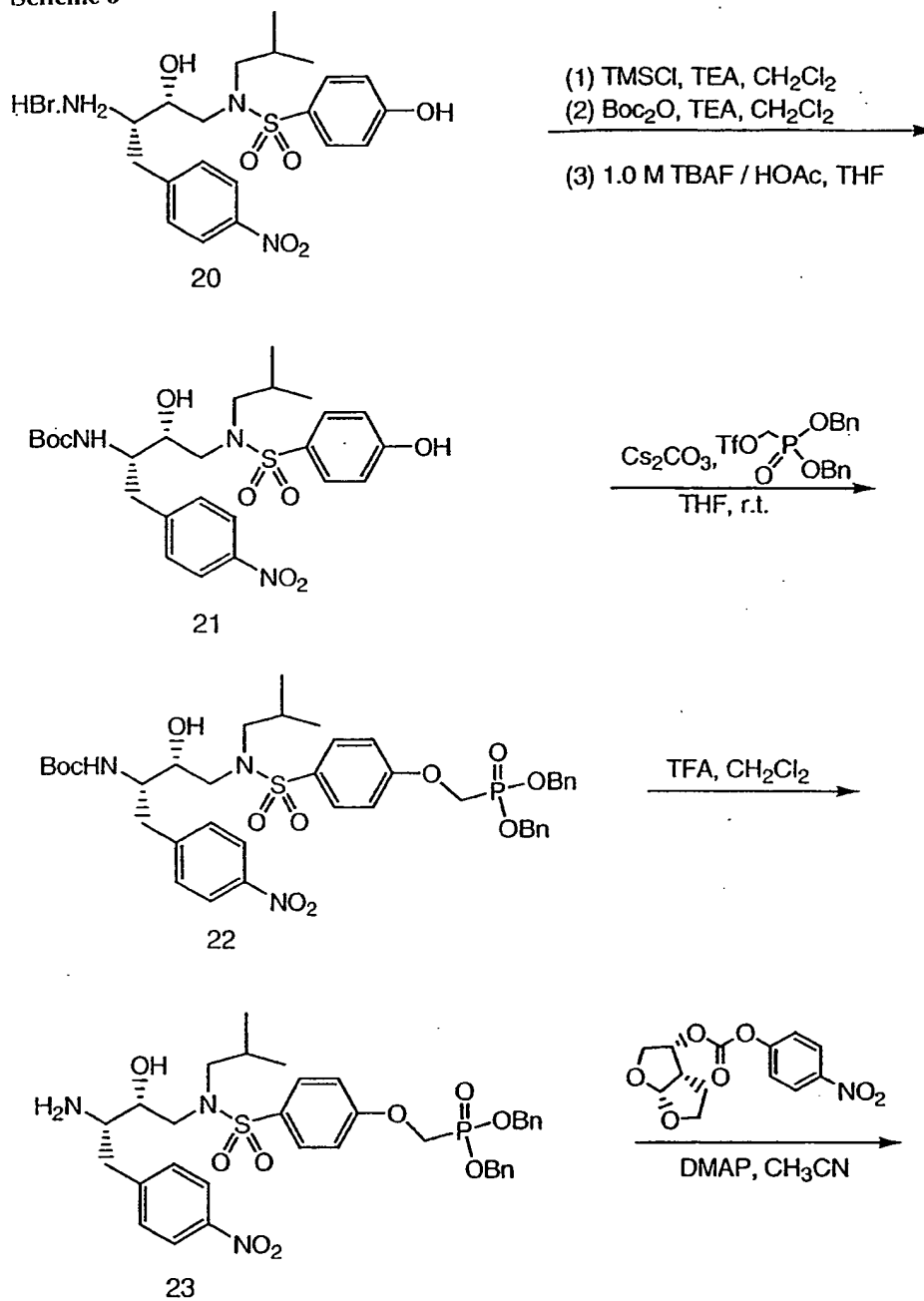


Scheme 5

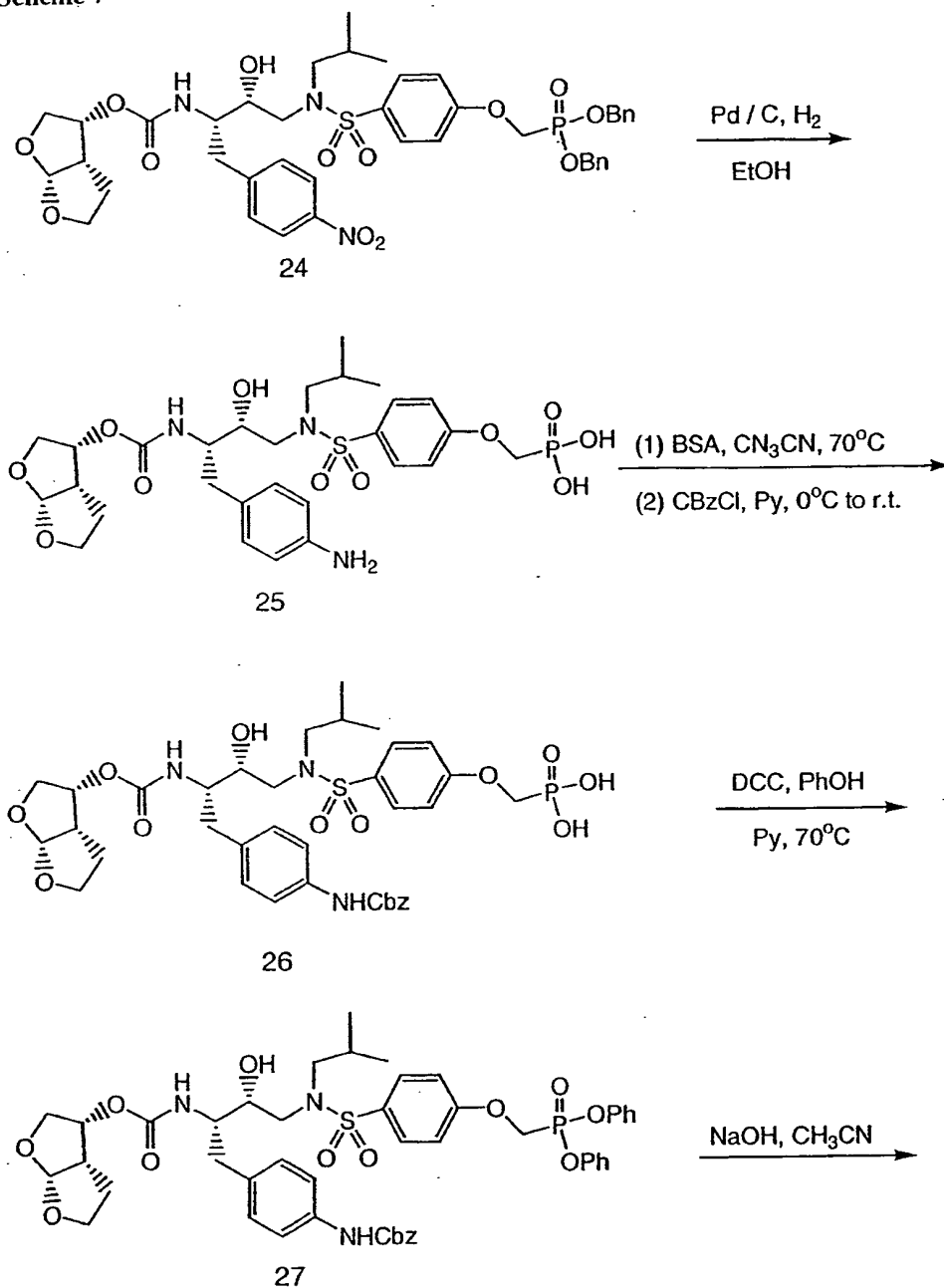




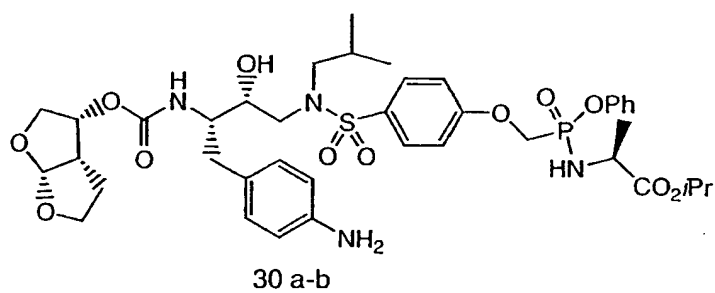
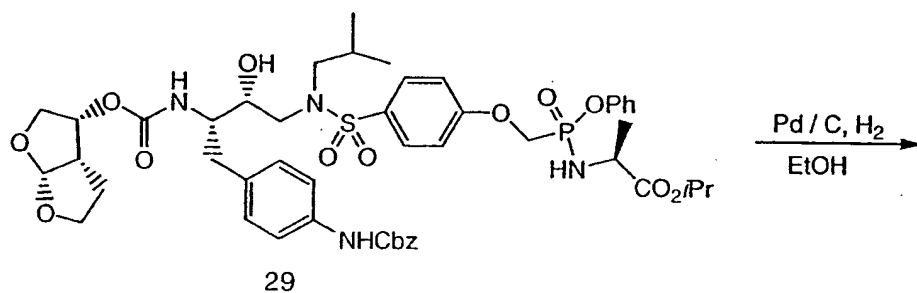
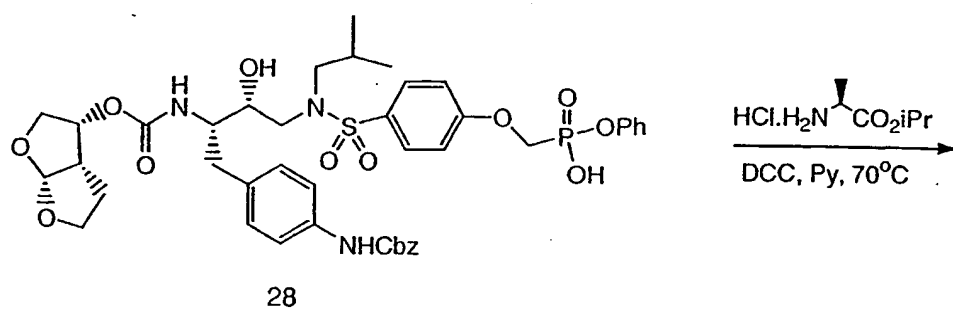
Scheme 6



Scheme 7

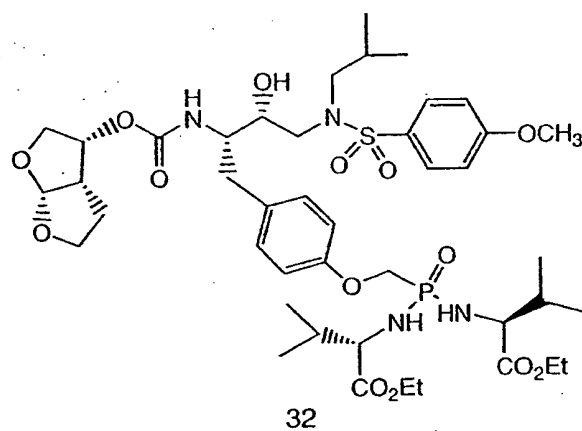
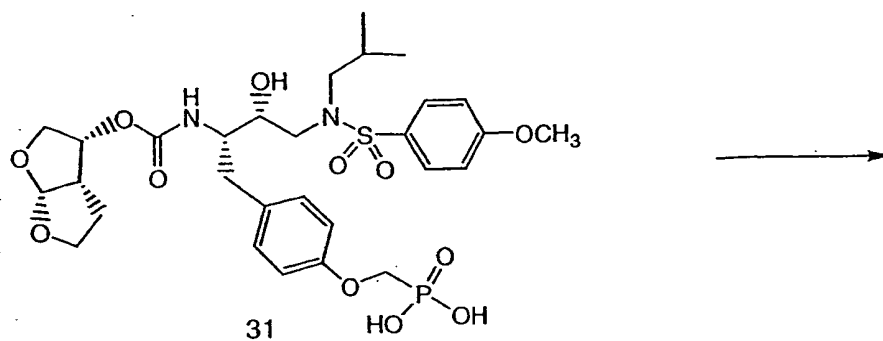


Scheme 8



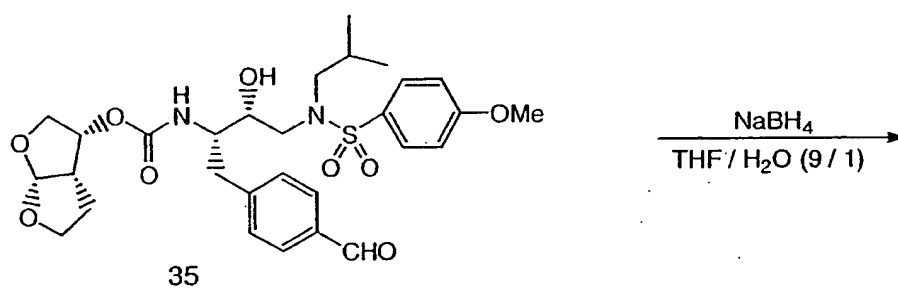
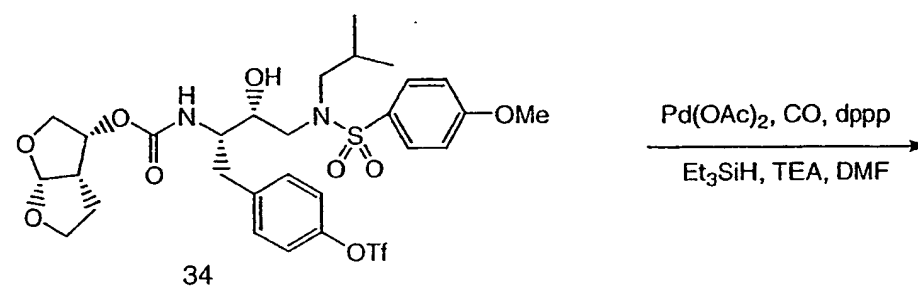
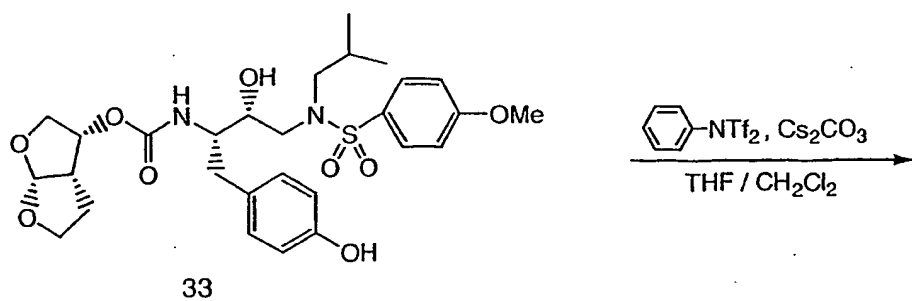
30a R = H, GS 77369  
30b R = Et, GS 77425

Scheme 9

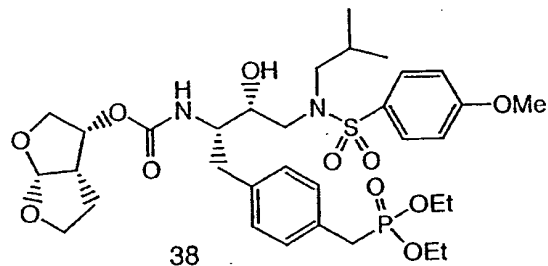
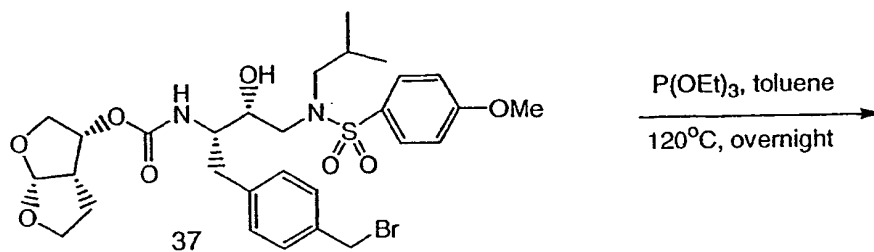
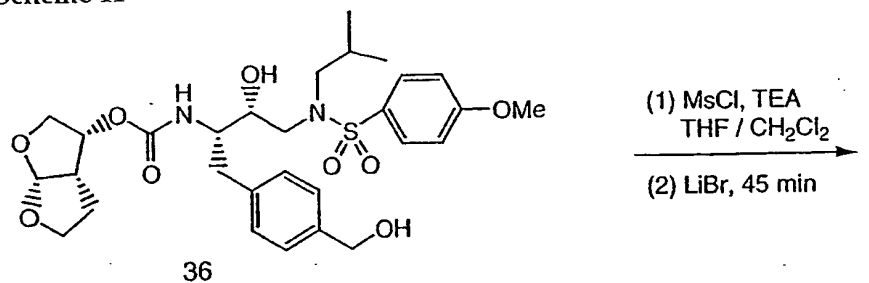


GS 17389

Scheme 10

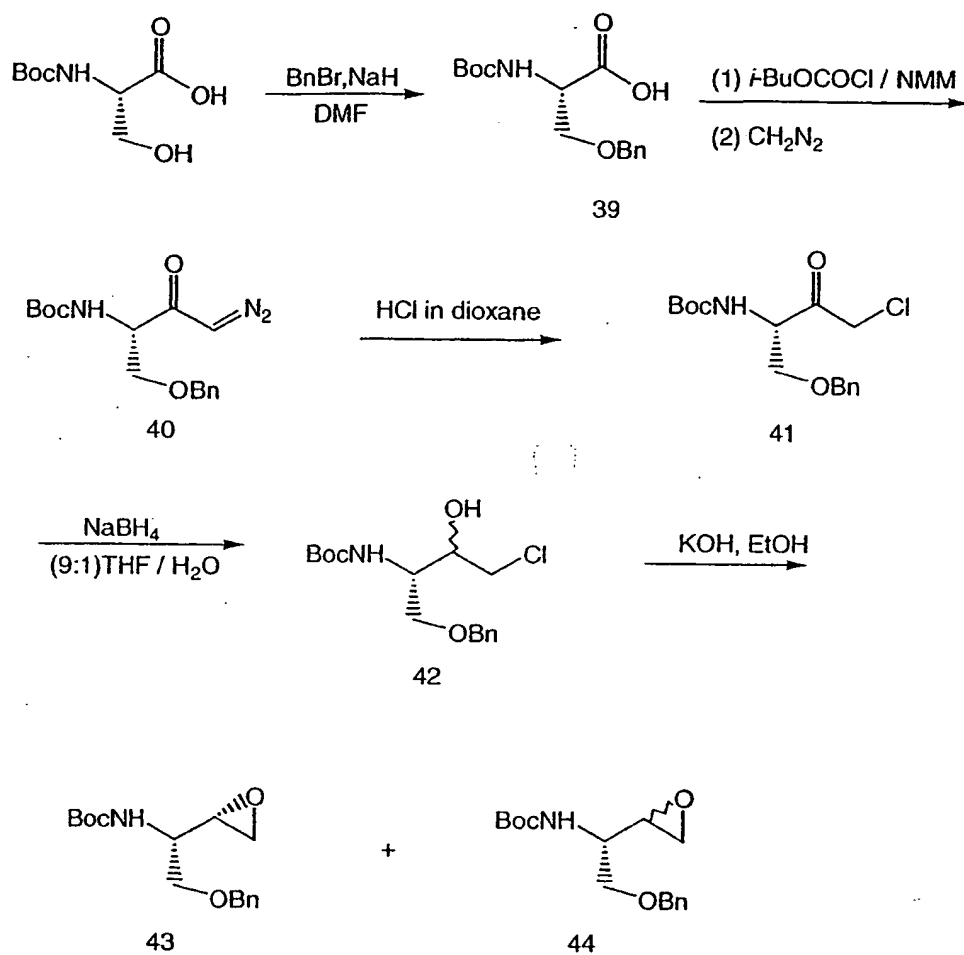


Scheme 11

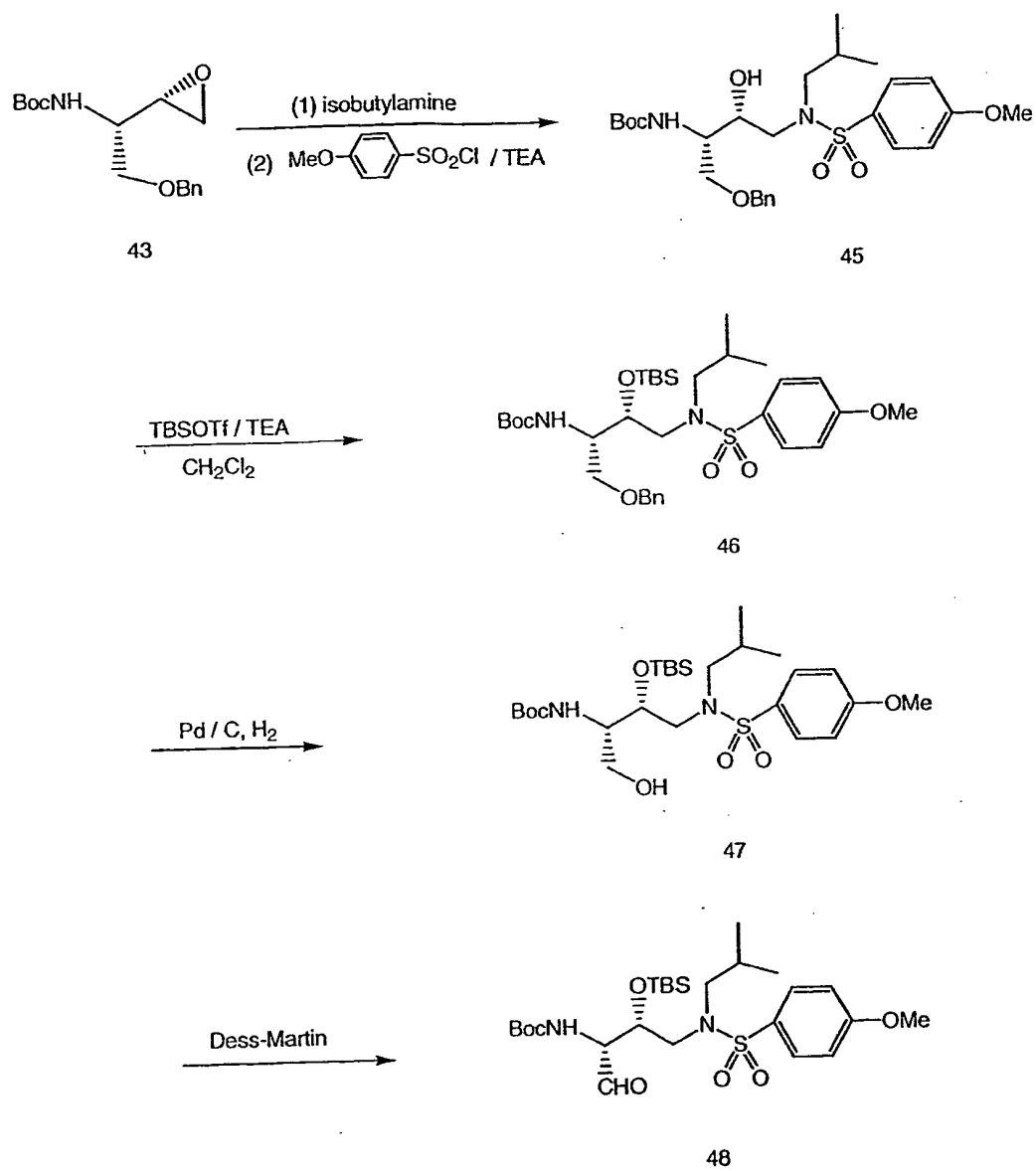


GS 191338

Scheme 12

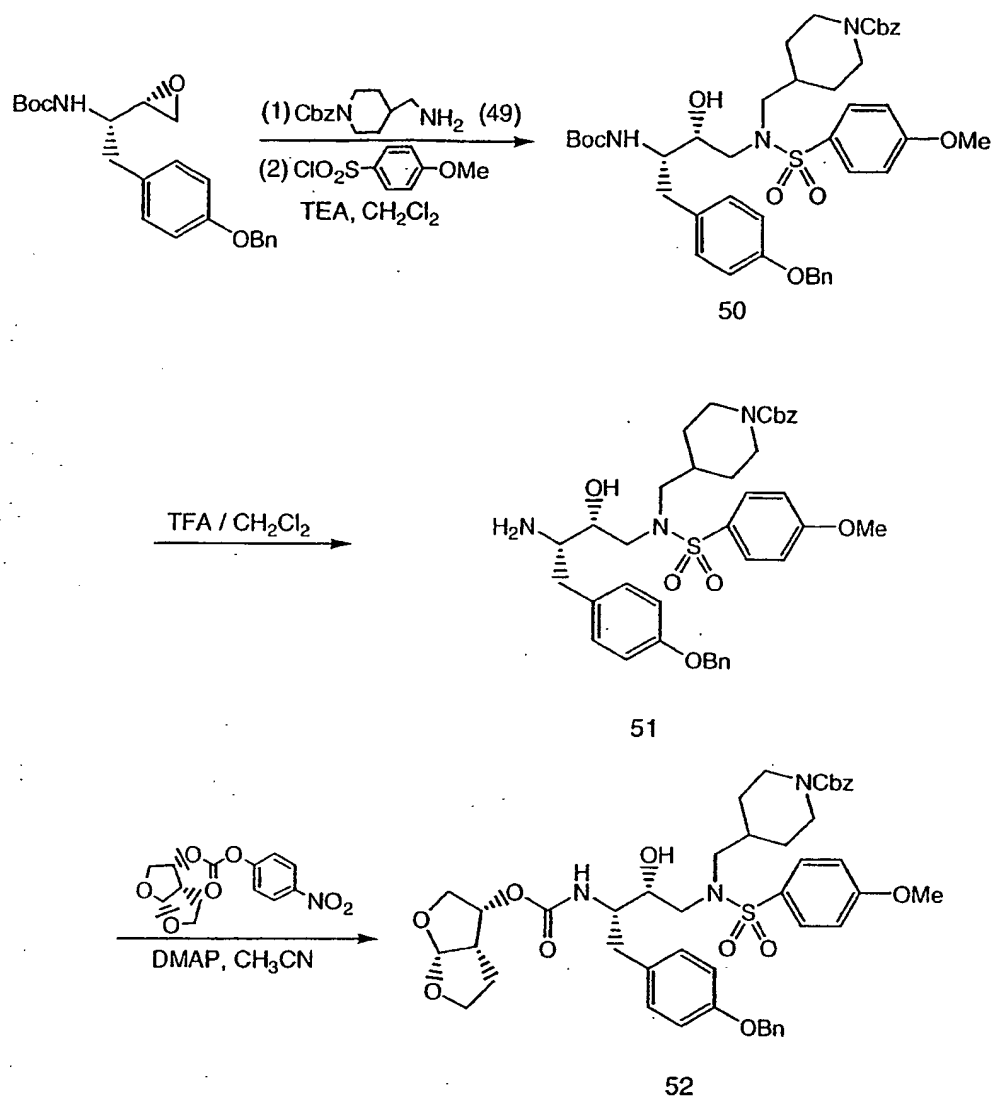


## Scheme 13



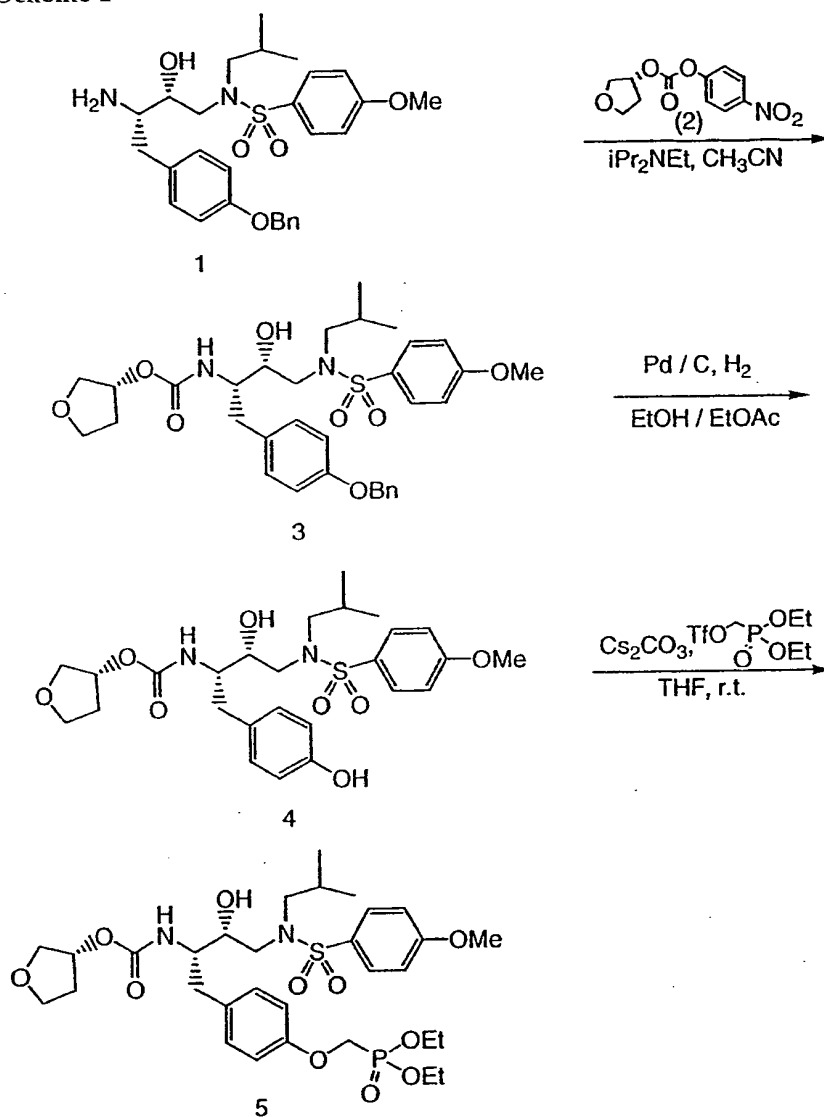


Scheme 14

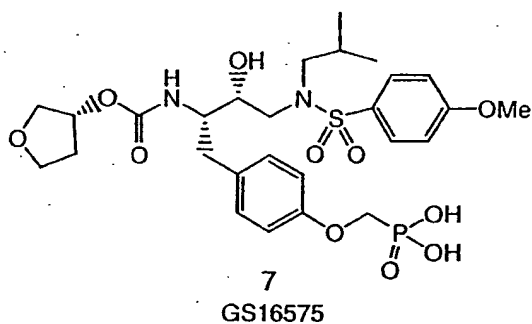
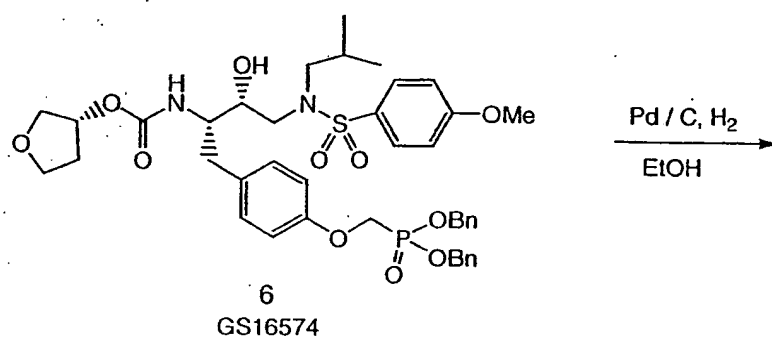
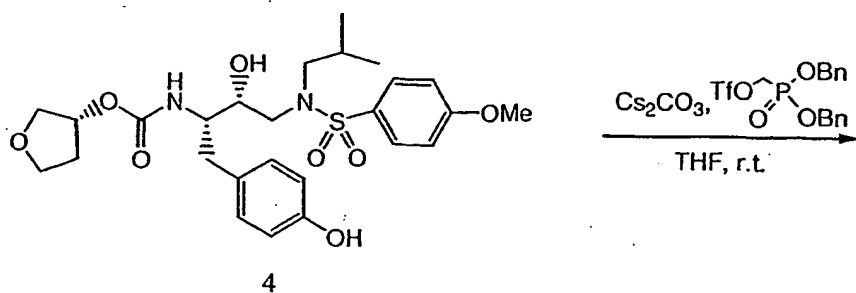


**Scheme Section I**

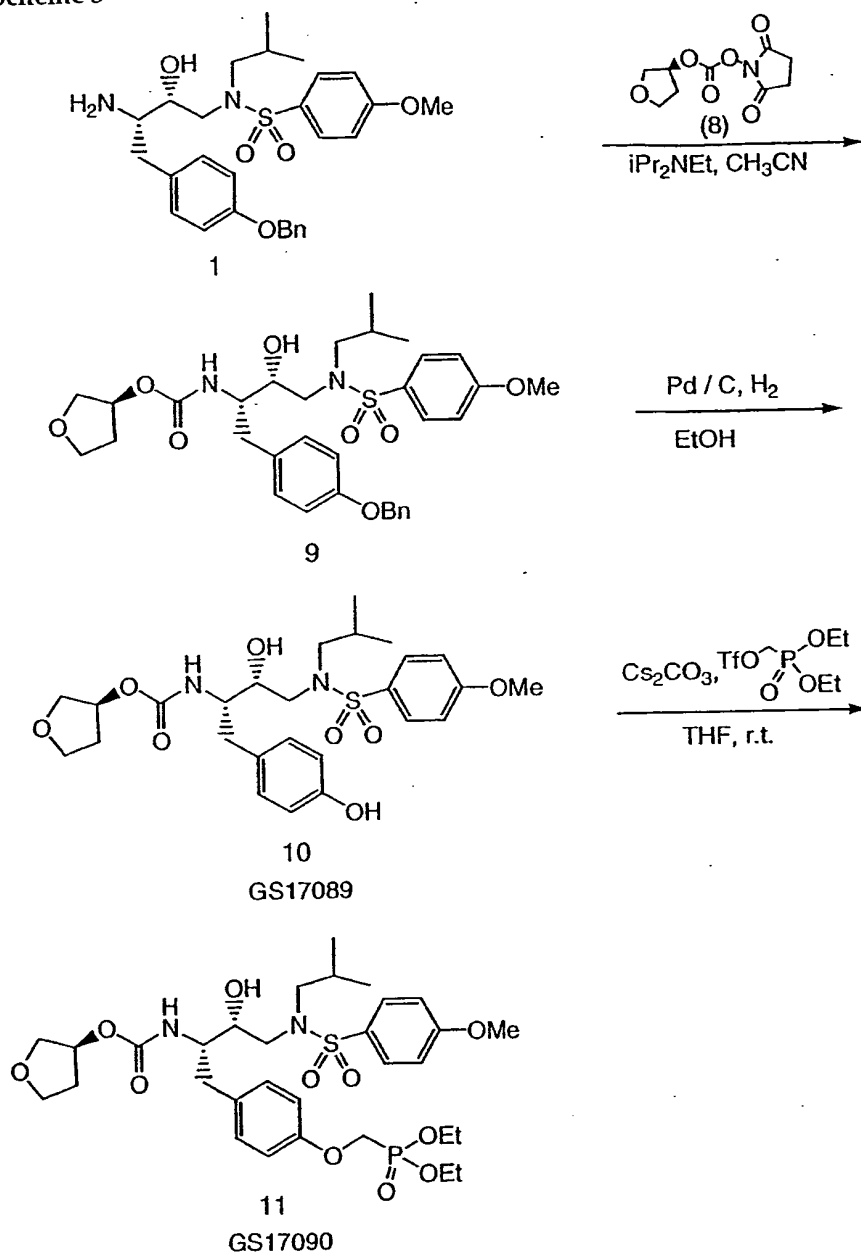
Schemes 1 to 3 are described in the examples.

**Scheme 1**

Scheme 2

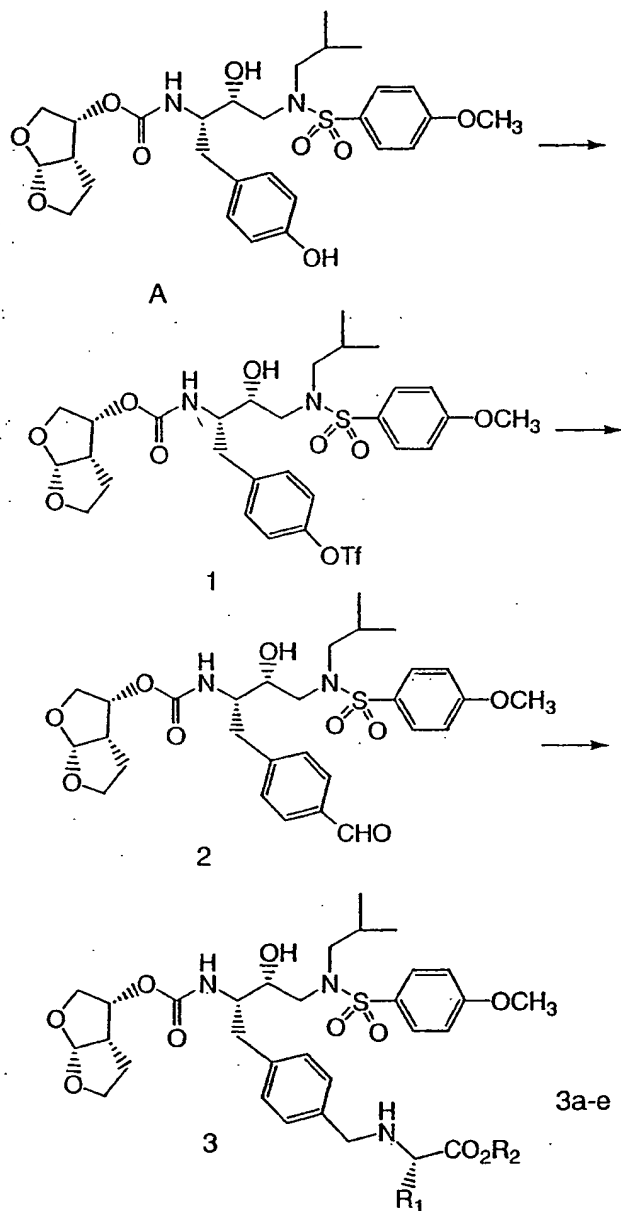


Scheme 3

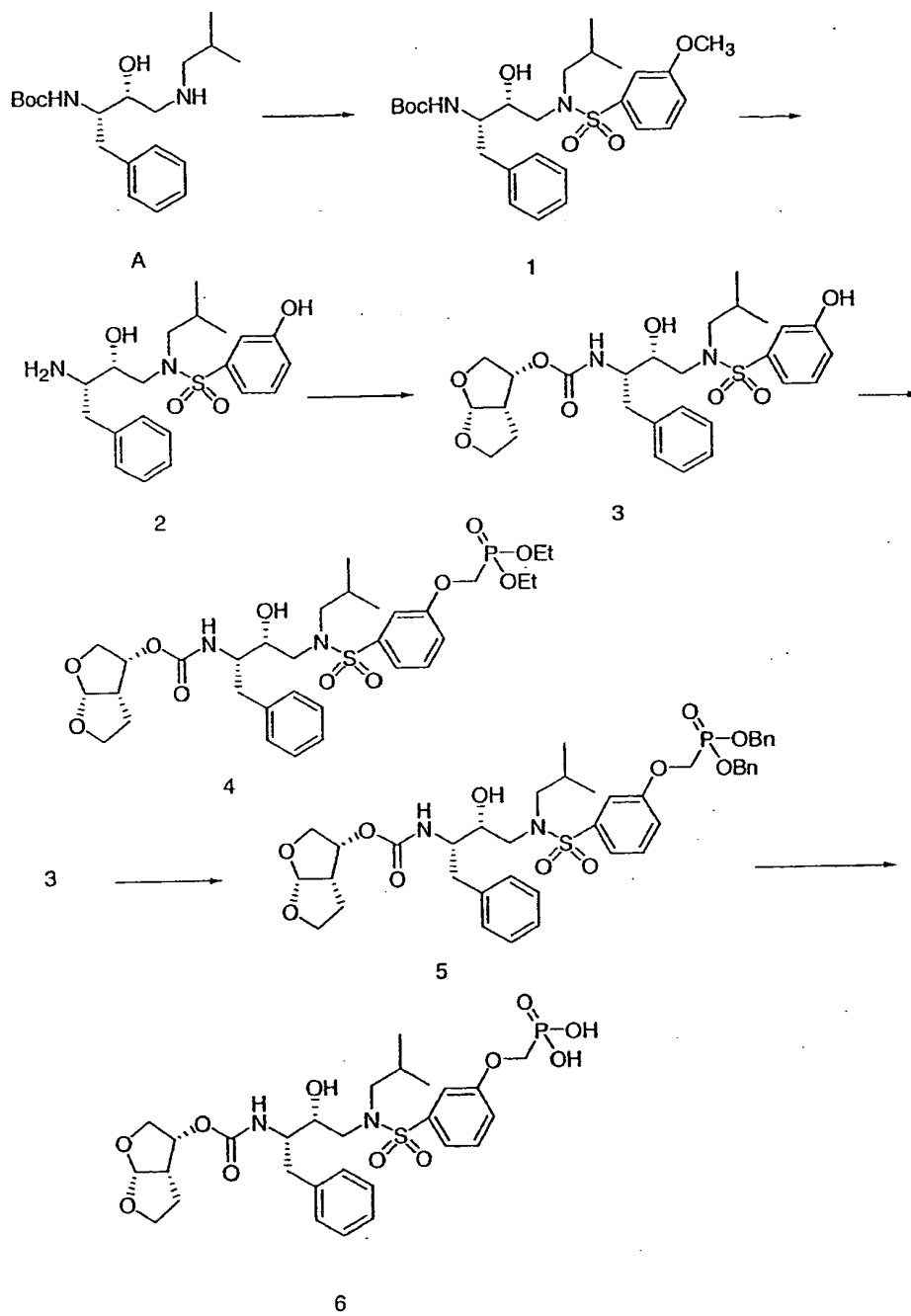


**Scheme Section I**

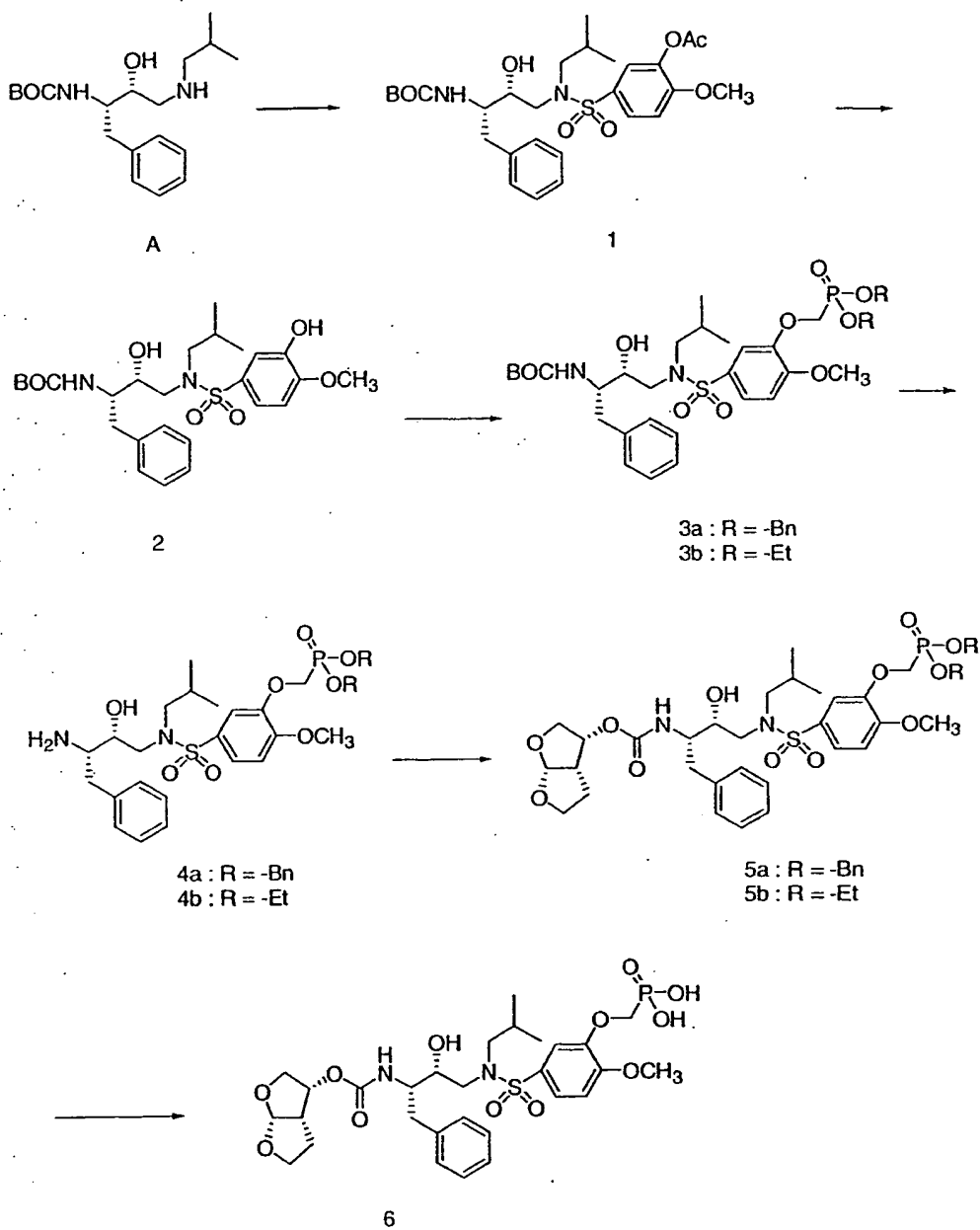
Schemes 1-4 are described in the examples.

**Scheme 1**

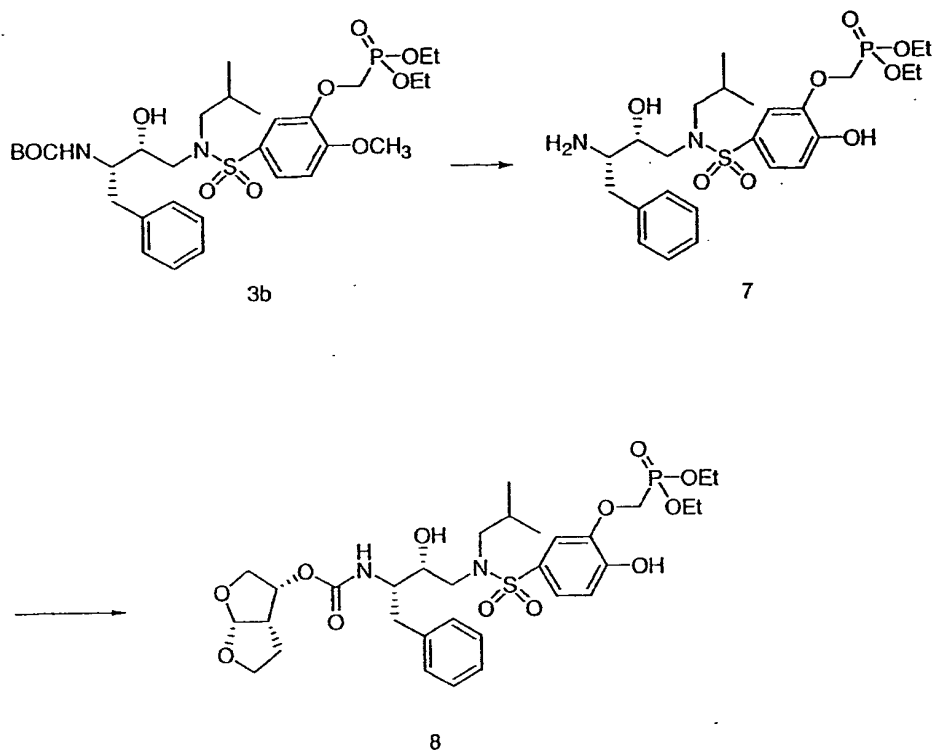
Scheme 2



Scheme 3



Scheme 4

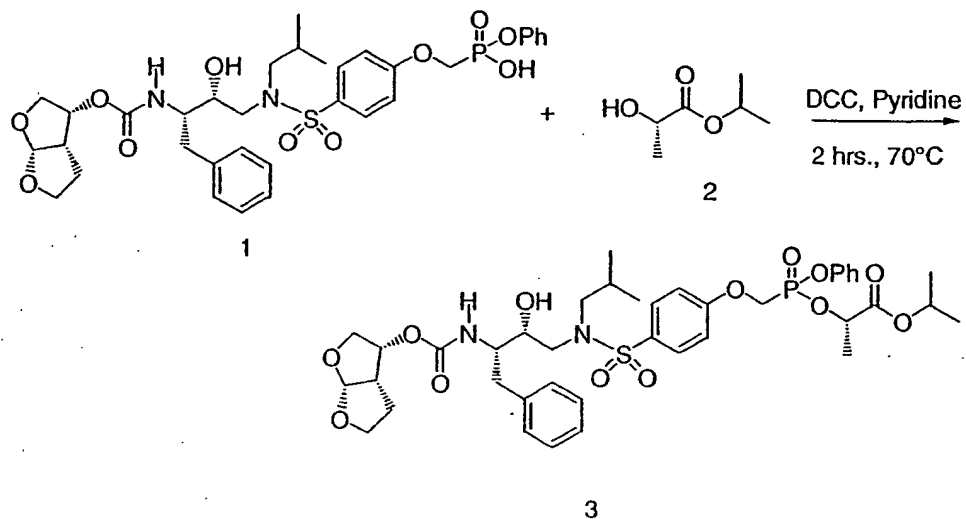




## Scheme Section K

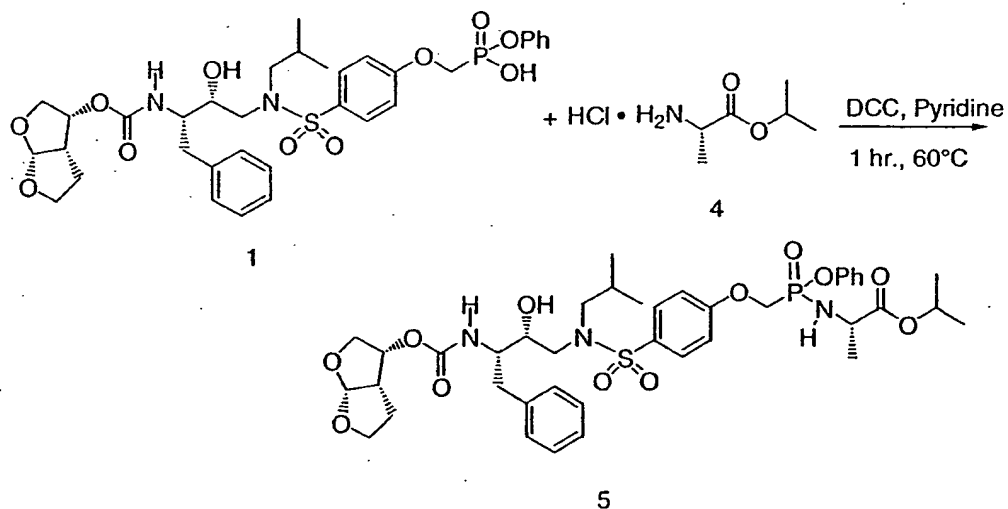
Schemes 1-9 are described in the examples.

## 5 Scheme 1

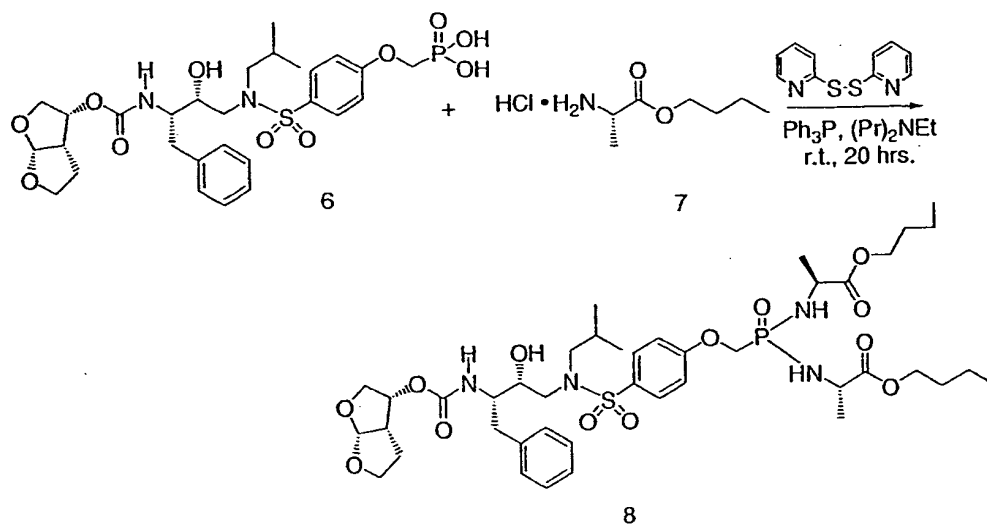


## Scheme 2

10

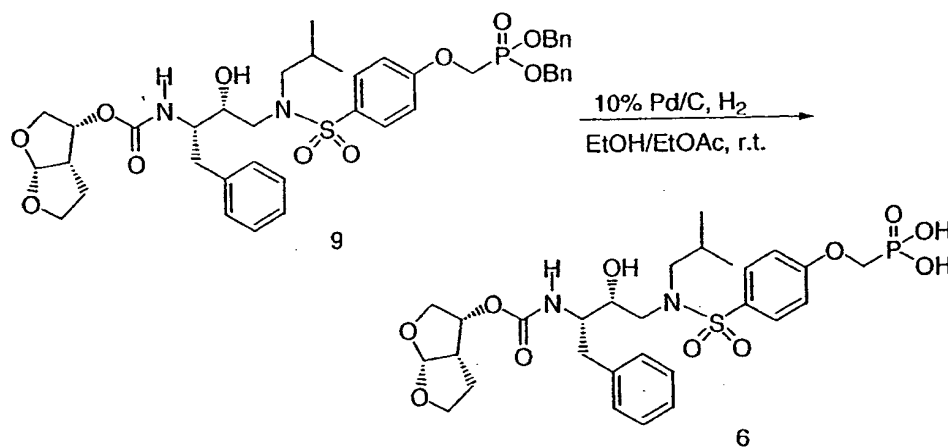


## Scheme 3

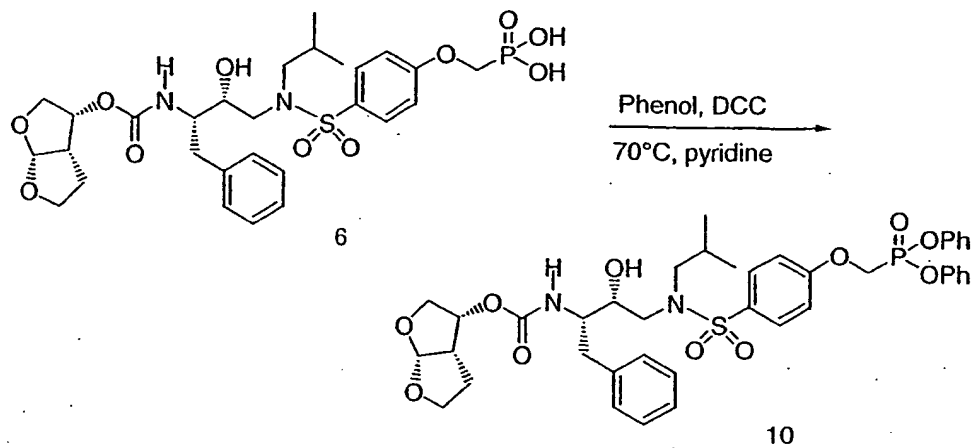


## Scheme 4

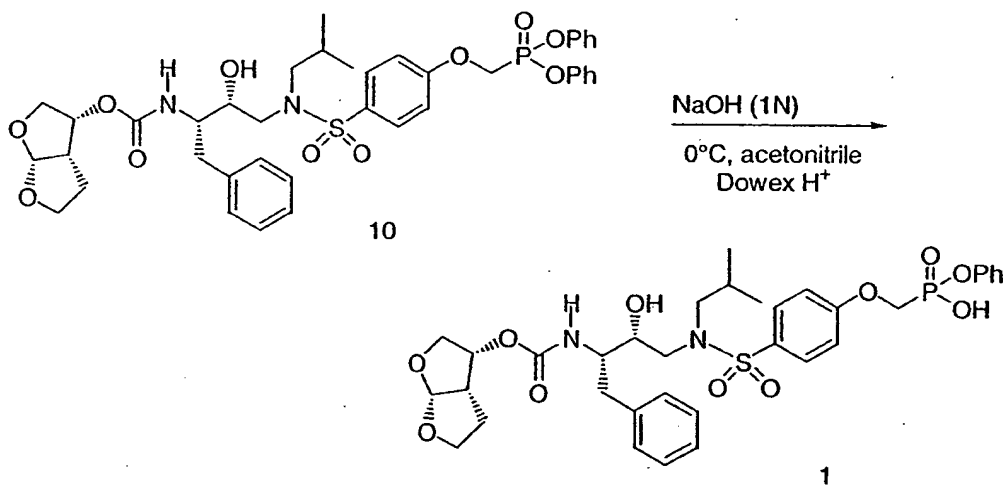
5



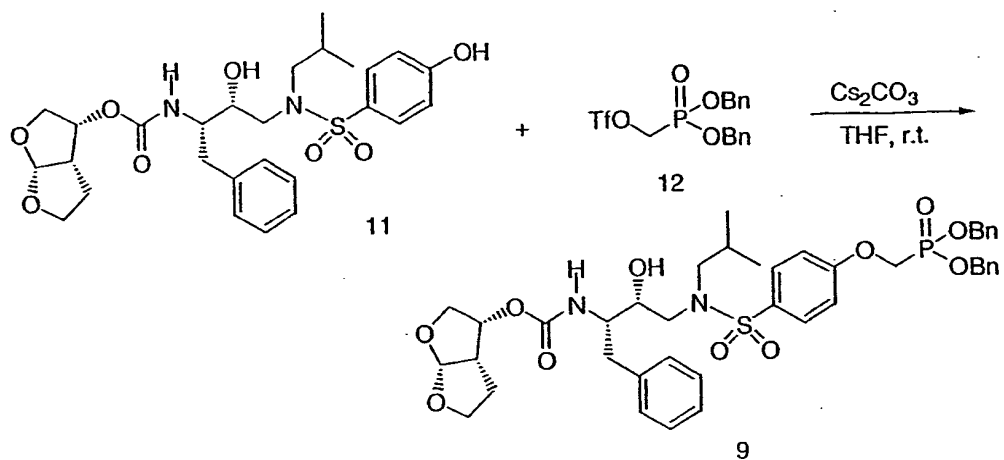
Scheme 5



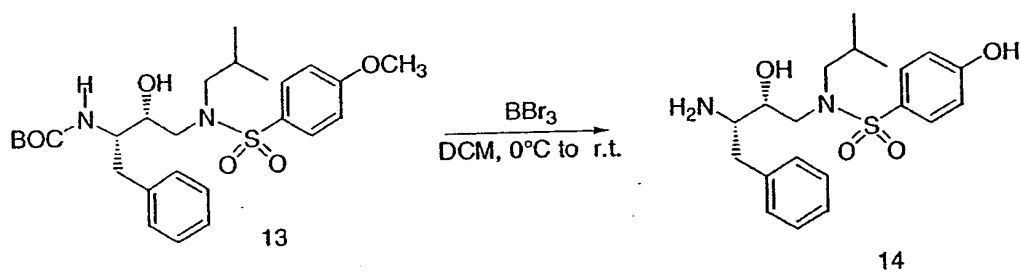
Scheme 6



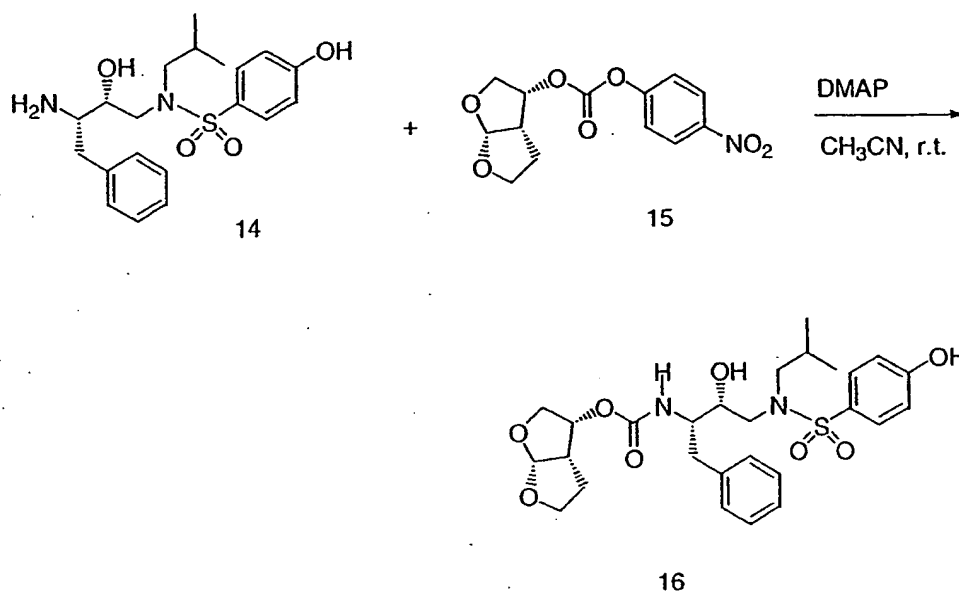
Scheme 7



5 Scheme 8

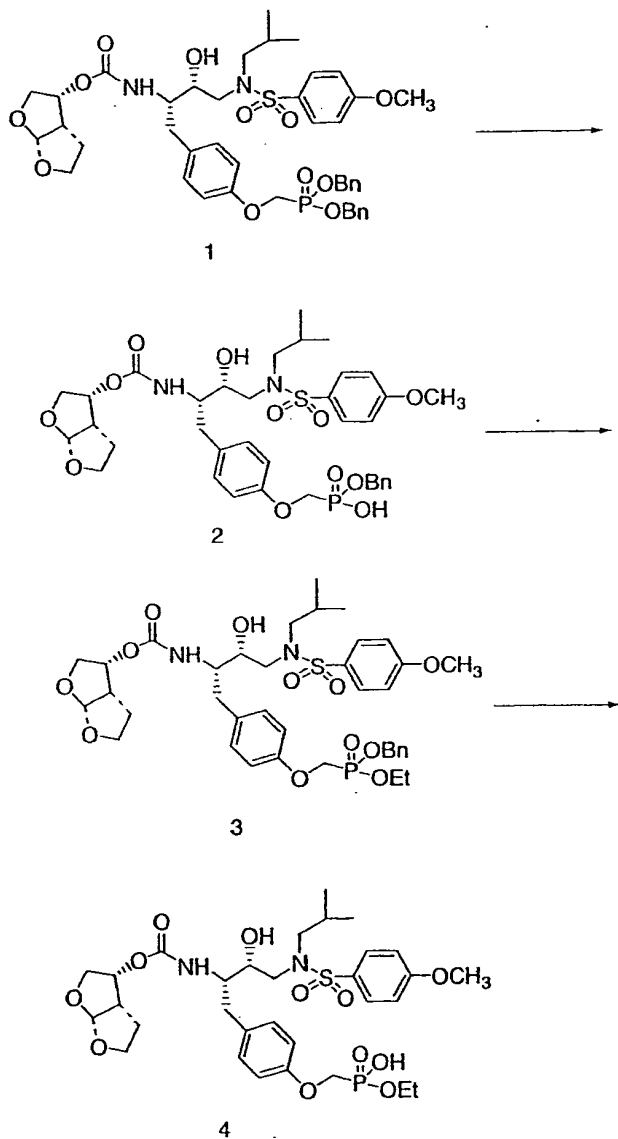


Scheme 9

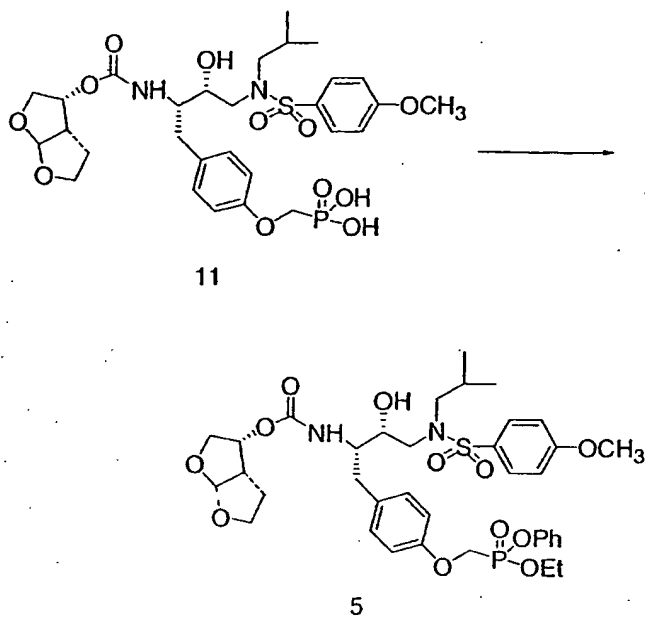


**Scheme Section L**

Schemes 1-9 are described in the examples.

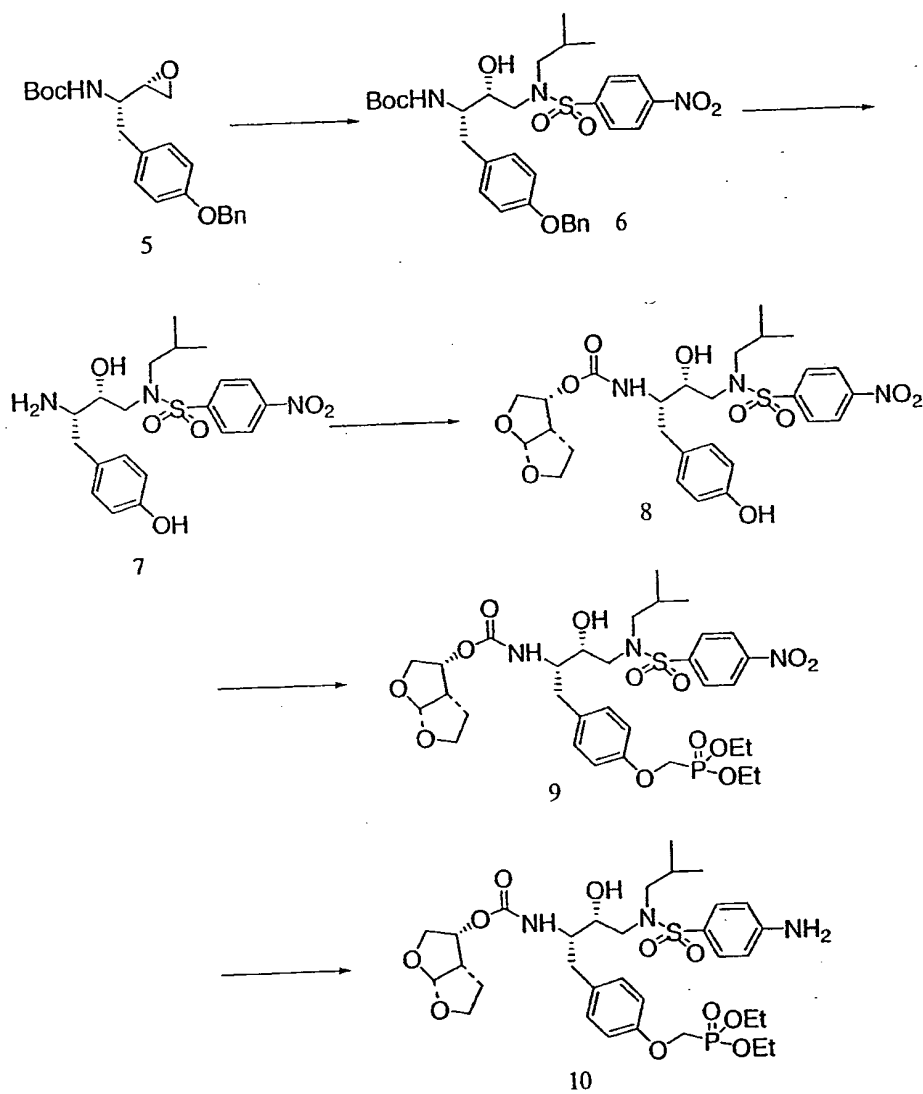
**Scheme 1****Synthesis of P1-Phosphonic ester**

## Scheme 2



## Scheme 3

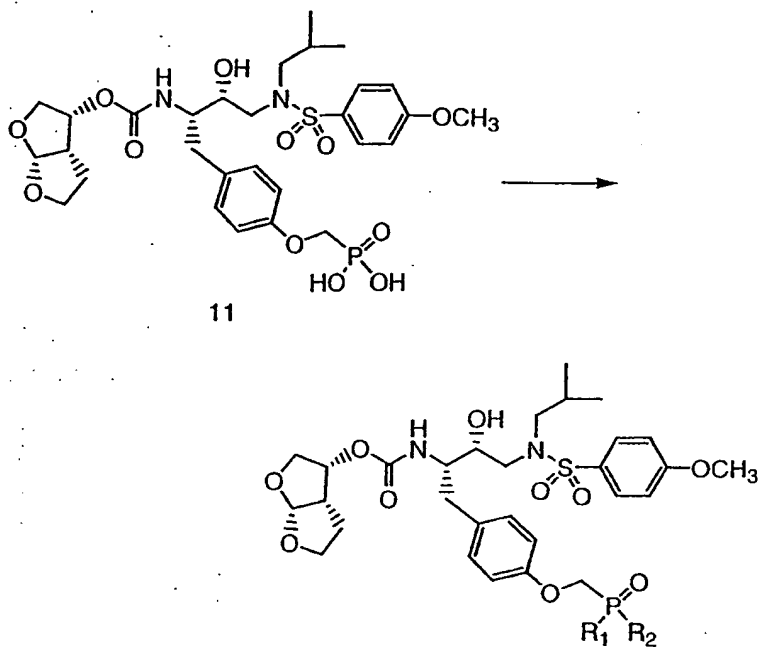
## Synthesis of P2'-Amino-P1-Phosphonic ester





## Scheme 4

## Synthesis of Bisamidates

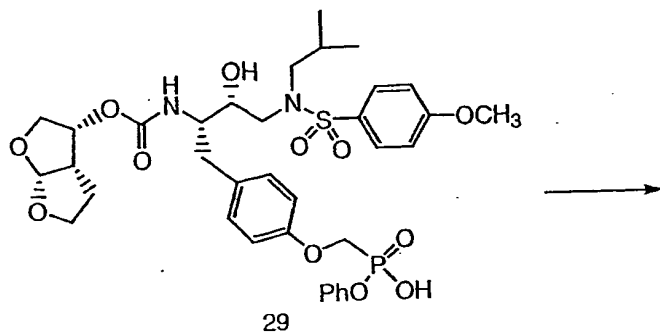


16 a,b,j and k

| Compound | R <sub>1</sub> | R <sub>2</sub> |
|----------|----------------|----------------|
| 16a      | Gly-Et         | Gly-Et         |
| 16b      | Gly-Bu         | Gly-Bu         |
| 16j      | Phe-Bu         | Phe-Bu         |
| 16k      | NHEt           | NHEt           |

## Scheme 5

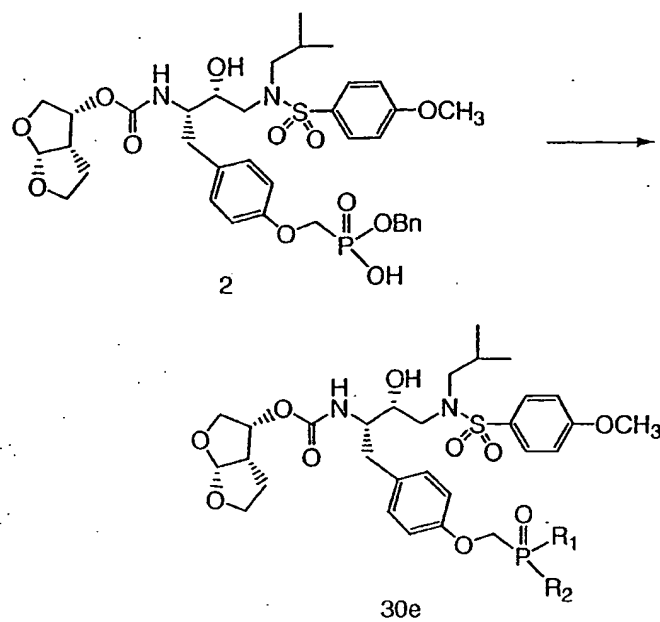
## Synthesis of Monoamidates



5

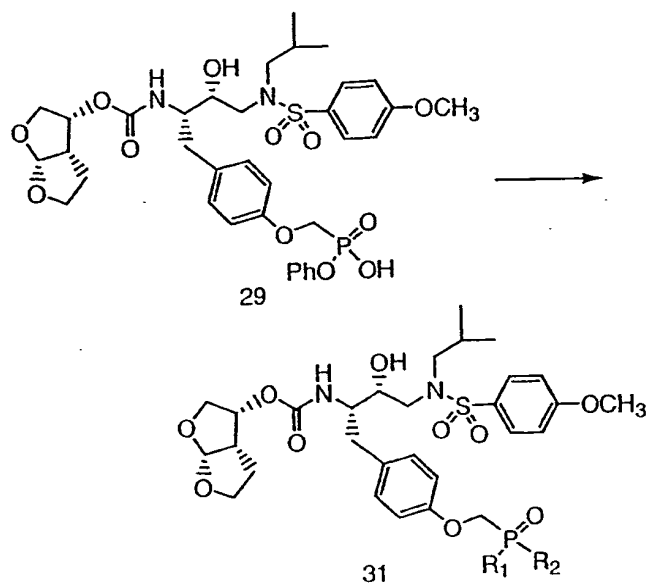
| Compound | $R_1$ | $R_2$       |
|----------|-------|-------------|
| 30a      | OPh   | Ala-Me      |
| 30b      | OPh   | Ala-Et      |
| 30c      | OPh   | (D)-Ala-iPr |
| 30d      | OPh   | Ala-Bu      |
| 30e      | OBn   | Ala-Et      |

Scheme 6



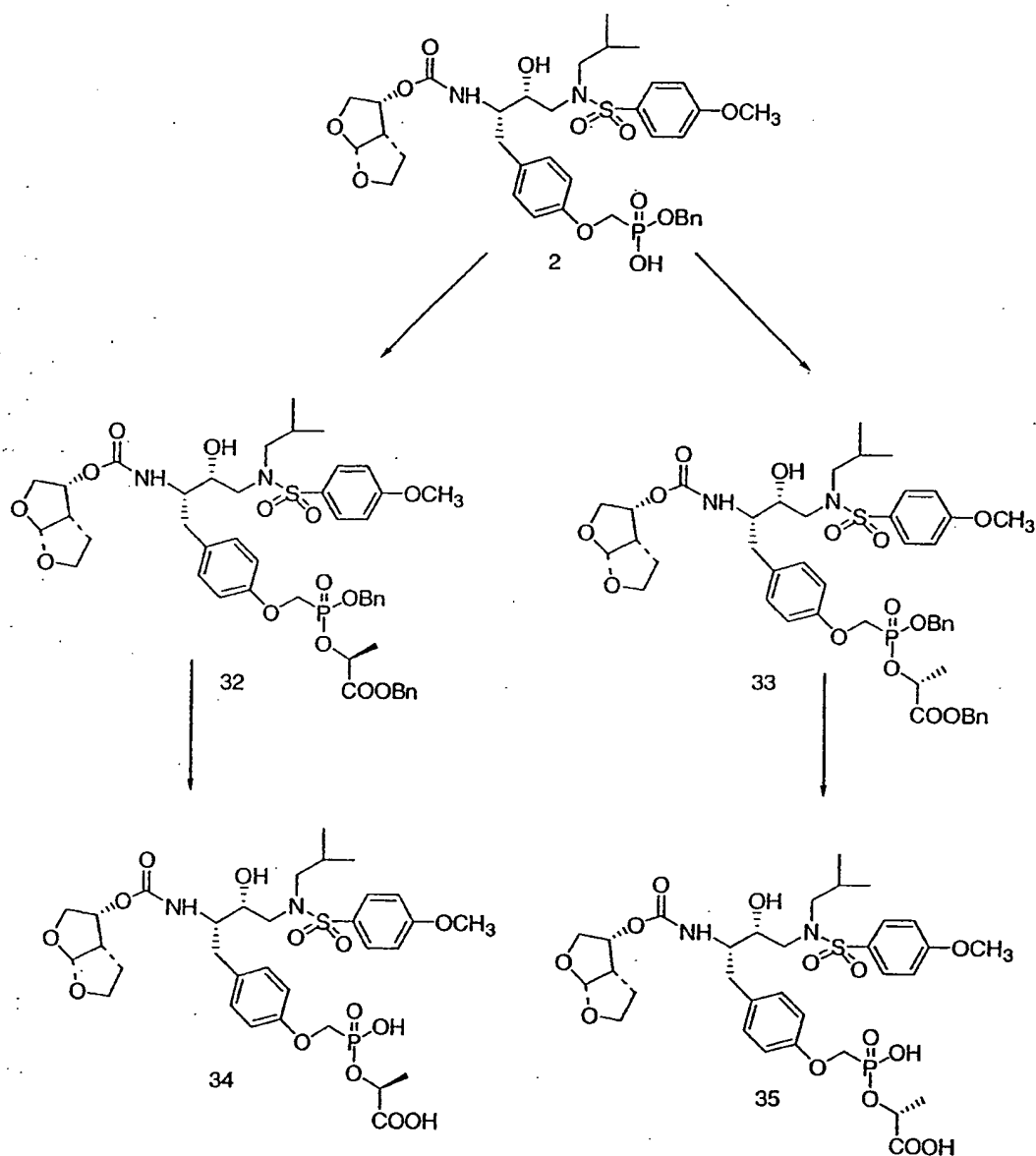
## Scheme 7

## Synthesis of Lactates



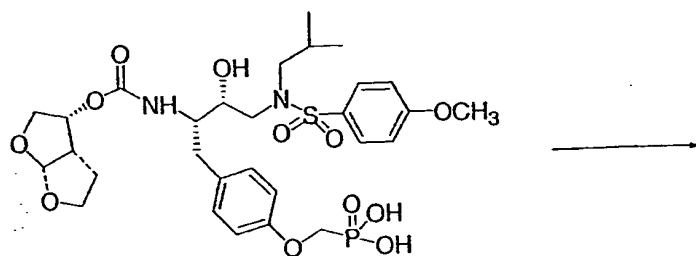
| Compound | R <sub>1</sub> | R <sub>2</sub> |
|----------|----------------|----------------|
| 31a      | OPh            | Lac-iPr        |
| 31b      | OPh            | Lac-Et         |
| 31c      | OPh            | Lac-Bu         |
| 31d      | OPh            | (R)-Lac-Me     |
| 31e      | OPh            | (R)-Lac-Et     |

Scheme 8

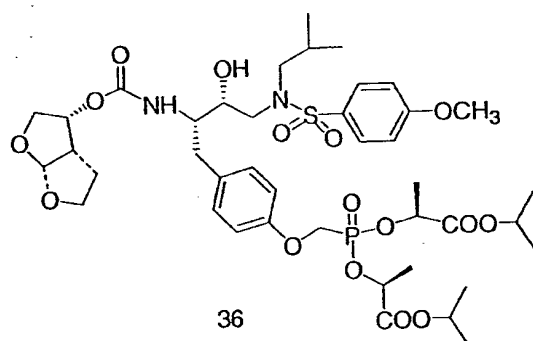


## Scheme 9

## Synthesis of Bislactate



11



36

## Examples

The following Examples refer to the Schemes.

- 5 Some Examples have been performed multiple times. In repeated Examples, reaction conditions such as time, temperature, concentration and the like, and yields were within normal experimental ranges. In repeated Examples where significant modifications were made, these have been noted where the results varied significantly from those described. In Examples where different starting materials were used, these are noted. When the repeated
- 10 Examples refer to a "corresponding" analog of a compound, such as a "corresponding ethyl ester", this intends that an otherwise present group, in this case typically a methyl ester, is taken to be the same group modified as indicated.

### Example Section A

15 Example 1

- Diazo ketone 1: To a solution of N-tert-Butoxycarbonyl-O-benzyl-L-tyrosine (11 g, 30 mmol, Fluka) in dry THF (55 mL) at -25-30°C (external bath temperature) was added isobutylchloroformate (3.9 mL, 30 mmol) followed by the slow addition of N.methylmorpholine (3.3 mL, 30 mmol). The mixture was stirred for 25 min, filtered while
- 20 cold, and the filter cake was rinsed with cold (0°C) THF (50 mL). The filtrate was cooled to -25°C and diazomethane (~50 mmol, generated from 15 g Diazald according to Aldrichimica Acta 1983, 16, 3) in ether (~150 mL) was poured into the mixed anhydride solution. The reaction was stirred for 15 min and was then placed in an icebath at 0°C, allowing the bath to warm to room temperature while stirring overnight for 15 h. The solvent was evaporated
- 25 under reduced pressure and the residue was dissolved in EtOAc, washed with water, saturated NaHCO<sub>3</sub>, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated to a pale yellow solid. The crude solid was slurried in hexane, filtered, and dried to afford the diazo ketone (10.9 g, 92%) which was used directly in the next step.

30 Example 2

- Chloroketone 2: To a suspension of diazoketone 1 (10.8 g, 27 mmol) in ether (600 mL) at 0°C was added 4M HCl in dioxane (7.5 mL, 30 mmol). The solution was removed from the cooling bath, and allowed to warm to room temperature at which time the reaction was stirred 1 h. The reaction solvent was evaporated under reduced pressure to give a solid residue that

was dissolved in ether and passed through a short column of silica gel. The solvent was evaporated to afford the chloroketone (10.7 g, 97%) as a solid.

### Example 3

5 Chloroalcohol 3: To a solution of chloroketone 2 (10.6 g, 26 mmol) in THF (90 mL) was added water (10 mL) and the solution was cooled to 3-4°C (internal temperature). A solution of NaBH<sub>4</sub> (1.5 g, 39 mmol) in water (5 mL) was added dropwise over a period of 10 min. The mixture was stirred for 1h at 0°C and saturated KHSO<sub>4</sub> was slowly added until the pH<4 followed by saturated NaCl. The organic phase was washed with saturated NaCl, dried  
10 (MgSO<sub>4</sub>) filtered and evaporated under reduced pressure. The crude product consisted of a 70:30 mixture of diastereomers by HPLC analysis (mobile phase, 77:25-CH<sub>3</sub>CN:H<sub>2</sub>O; flow rate: 1 mL/min; detection: 254 nm; sample volume: 20 µL; column: 5µ C18, 4.6X250 mm, Varian; retention times: major diastereomer 3, 5.4 min, minor diastereomer 4, 6.1 min). The residue was recrystallized from EtOAc/hexane twice to afford the chloro alcohol 3 (4.86g,  
15 >99% diastereomeric purity by HPLC analysis) as a white solid.

### Example 4

Epoxide 5: A solution of chloroalcohol 3 (4.32 g, 10.6 mmol) in EtOH (250 mL) and THF (100 mL) was treated with K<sub>2</sub>CO<sub>3</sub> (4.4g, 325 mesh, 31.9 mmol) and the mixture was stirred  
20 for at room temperature for 20h. The reaction mixture was filtered and was evaporated under reduced pressure. The residue was partitioned between EtOAc and water and the organic phase was washed with saturated NaCl, dried (MgSO<sub>4</sub>), filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel to afford the epoxide (3.68 g, 94%) as a white solid.

25

### Example 5

Sulfonamide 6: To a suspension of epoxide 5 (2.08 g, 5.6 mmol) in 2-propanol (20 mL) was added isobutylamine (10.7 mL, 108 mmol) and the solution was refluxed for 30 min. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub>  
30 (20 mL) and cooled to 0°C. N,N'-diisopropylethylamine (1.96 mL, 11.3 mmol) was added followed by the addition of 4-methoxybenzenesulfonyl chloride (1.45 g, 7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the solution was stirred for 40 min at 0°C, warmed to room temperature and



evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexane to give the sulfonamide (2.79 g, 81%) as a small white needles: mp 122-  
5 124°C (uncorrected).

#### Example 6

Carbamate 7: A solution of sulfonamide 6 (500 mg, 0.82 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at 0°C was treated with trifluoroacetic acid (5 mL). The solution was stirred at 0°C for 30 min and  
10 was removed from the cold bath stirring for an additional 30 min. Volatiles were evaporated under reduced pressure and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The aqueous phase was extracted twice with  $\text{CH}_2\text{Cl}_2$  and the combined organic extracts were washed with saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure. The residue was dissolved in  $\text{CH}_3\text{CN}$  (5 mL) and was treated with (3R,  
15 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (263 mg, 0.89 mmol, prepared according to Ghosh et al., J. Med. Chem. 1996, 39, 3278.) and N,N-dimethylaminopyridine (197 mg, 1.62 mmol). After stirring for 1.5h at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 5% citric acid. The organic phase was washed twice with 1%  $\text{K}_2\text{CO}_3$ ,  
20 and then was washed with saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (1/1 - EtOAc/hexane) affording the carbamate (454 mg, 83%) as a solid: mp 128-129°C (MeOH, uncorrected).

#### 25 Example 7

Phenol 8: A solution of carbamate 7 (1.15 g, 1.7 mmol) in EtOH (50 mL) and EtOAc (20 mL) was treated with 10% Pd/C (115 mg) and was stirred under  $\text{H}_2$  atmosphere (balloon) for 18h. The reaction solution was purged with  $\text{N}_2$ , filtered through a 0.45  $\mu\text{M}$  filter and was evaporated under reduced pressure to afford the phenol as a solid that contained residual  
30 solvent: mp 131-134°C (EtOAc/hexane, uncorrected).

#### Example 8

Dibenzylphosphonate 10: To a solution of dibenzylhydroxymethyl phosphonate (527 mg, 1.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was treated with 2,6-lutidine (300  $\mu\text{L}$ , 2.6 mmol) and the reaction flask was cooled to  $-50^\circ\text{C}$  (external temperature). Trifluoromethanesulfonic anhydride (360  $\mu\text{L}$ , 2.1 mmol) was added and the reaction mixture was stirred for 15 min and then the cooling bath was allowed to warm to  $0^\circ\text{C}$  over 45 min. The reaction mixture was partitioned between ether and ice-cold water. The organic phase was washed with cold 1M  $\text{H}_3\text{PO}_4$ , saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure to afford triflate 9 (697 mg, 91%) as an oil which was used directly without any further purification. To a solution of phenol 8 (775 mg, 1.3 mmol) in THF (5 mL) was added  $\text{Cs}_2\text{CO}_3$  (423 mg, 1.3 mmol) and triflate 9 (710 mg, 1.7 mmol) in THF (2 mL). After stirring the reaction mixture for 30 min at room temperature additional  $\text{Cs}_2\text{CO}_3$  (423 mg, 1.3 mmol) and triflate (178 mg, 0.33 mmol) were added and the mixture was stirred for 3.5h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product was chromatographed on silica gel eluting (5% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the dibenzylphosphonate as an oil that solidified upon standing. The solid was dissolved in EtOAc, ether was added, and the solid was precipitated at room temperature overnight. After cooling to  $0^\circ\text{C}$ , the solid was filtered and washed with cold ether to afford the dibenzylphosphonate (836 mg, 76%) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.66 (d, 2H), 7.31 (s, 10H), 7.08 (d, 2H), 6.94 (d, 2H), 6.76 (d, 2H), 5.59 (d, 1H), 5.15-4.89 (m, 6H), 4.15 (d, 2H), 3.94-3.62 (m, 10H), 3.13-2.69 (m, 7H), 1.78 (m, 1H), 1.70-1.44 (m, 2H), 0.89-0.82 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  18.7; MS (ESI) 853 (M+H).

#### Example 9

Phosphonic acid 11: A solution of dibenzylphosphonate 10 (0.81 g) was dissolved in EtOH/EtOAc (30mL/10 mL), treated with 10% Pd/C (80 mg) and was stirred under  $\text{H}_2$  atmosphere (balloon) for 1.5h. The reaction was purged with  $\text{N}_2$ , and the catalyst was removed by filtration through celite. The filtrate was evaporated under reduced pressure and the residue was dissolved in MeOH and filtered with a 0.45  $\mu\text{m}$  filter. After evaporation of the filtrate, the residue was triturated with ether and the solid was collected by filtration to afford the phosphonic acid (634 mg, 99%) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.77 (d, 2H), 7.19 (d, 2H), 7.09 (d, 2H), 6.92 (d, 2H), 5.60 (d, 1H), 4.95 (m, 1H), 4.17 (d, 2H), 3.94 (m, 1H), 3.89

(s, 3H), 3.85-3.68 (m, 5H), 3.42 (dd, 1H), 3.16-3.06 (m, 2H), 2.96-2.84 (m, 3H), 2.50 (m, 1H), 2.02 (m, 1H), 1.58 (m, 1H), 1.40 (dd, 1H), 0.94 (d, 3H), 0.89 (d, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.2; MS (ESI) 671 (M-H).

5 Example 10

Diethylphosphonate 13: Triflate 12 was prepared from diethyl hydroxymethylphosphonate (2g, 11.9 mmol), 2,6-lutidine (2.1 mL, 17.9 mmol), and trifluoromethanesulfonic anhydride (2.5 mL, 14.9 mmol) as described for compound 9. To a solution of phenol 8 (60 mg, 0.10 mmol) in THF (2 mL) was added  $\text{Cs}_2\text{CO}_3$  (65mg, 0.20 mmol) and triflate 12 (45 mg, 0.15 mmol) in THF (0.25 mL). The mixture was stirred at room temperature for 2h and additional triflate (0.15 mmol) in THF (0.25 mL) was added. After 2h the reaction mixture was partitioned between EtOAc and saturated NaCl. The organic phase was dried ( $\text{MgSO}_4$ ), filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (EtOAc) to give a residue that was purified by chromatography on silica gel (5% 2-propanol /  $\text{CH}_2\text{Cl}_2$ ) to afford the diethylphosphonate as a foam:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.66 (d, 2H), 7.10 (d, 2H), 6.94 (d, 2H), 6.82 (d, 2H), 5.60 (d, 1H), 4.97 (d, 2H), 4.23-4.13 (m, 6H), 3.93-3.62 (m, 10H), 3.12-2.68 (m, 7H), 1.84-1.44 (m, 3H), 1.31 (t, 6H), 0.88-0.82 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.7; MS (ESI) 729 (M+H).

20 Example 11

Diphenylphosphonate 14: To a solution of 11 (100mg, 0.15 mmol) and phenol (141 mg, 1.5 mmol) in pyridine (1.5 mL) was added N, N-diisopropylcarbodiimide (50  $\mu\text{L}$ , 0.38 mmol). The solution was stirred for 31h at room temperature and for 20h at 50°C. The solvent was evaporated under reduced pressure and the residue was purified by chromatography on silica gel eluting (EtOAc) to provide diphenylphosphonate 14 (16 mg) as a foam:  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.9; MS (ESI) 847 (M+Na).

Example 12

Bis-Poc-phosphonate 15: To a solution of 11 (50 mg, 0.74 mmol) and isopropylchloromethyl carbonate (29 mg, 0.19 mmol) in DMF (0.5 mL) was added triethylamine (26  $\mu\text{L}$ , 0.19 mmol) and the solution was heated at 70°C (bath temperature) for 4.5h. The reaction was concentrated under reduced pressure and the residue was purified by preparative layer

chromatography (2% 2-propanol/ CH<sub>2</sub>Cl<sub>2</sub>) to afford 15 (7 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (d, 2H), 7.15 (d, 2H); 7.01 (d, 2H), 6.93 (d, 2H), 5.80-5.71 (m, 4H), 5.67 (d, 1H), 5.07-4.87 (m, 4H), 4.35 (d, 2H), 4.04-3.68 (m, 10H), 3.13 (dd, 1H), 3.04-2.90 (m, 5H), 2.79 (dd, 1H), 1.88-1.50 (m, 3H+H<sub>2</sub>O peak), 1.30 (m, 12H), 0.93 (d, 3H), 0.88 (d, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 19.6.

5

### Example 13

Synthesis of Bisamidates 16a-j. Representative Procedure, Bisamidate 16f: A solution of phosphonic acid 11 (100 mg, 0.15 mmol) and (S)-2-aminobutyric acid butyl ester hydrochloride (116 mg, 0.59 mmol) was dissolved in pyridine (5 mL) and the solvent was

10 distilled under reduced pressure at 40-60°C. The residue was treated with a solution of Ph<sub>3</sub>P (117 mg, 0.45 mmol) and 2,2'-dipyridyl disulfide (98 mg, 0.45 mmol) in pyridine (1 mL) stirring for 20h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>). The purified product was suspended in ether and was evaporated under reduced pressure to afford

15 bisamidate 16f (106 mg, 75%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, 2H), 7.15 (d, 2H), 7.01 (d, 2H), 6.87 (d, 2H), 5.67 (d, 1H), 5.05 (m, 1H), 4.96 (d, 1H), 4.19-3.71 (m overlapping s, 18H), 3.42 (t, 1H), 3.30 (t, 1H), 3.20 (dd, 1H), 3.20-2.97 (m, 4H), 2.80 (dd, 2H), 1.87-1.54 (m, 19H), 1.42-1.35 (4H), 0.97-0.88 (m, 18H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.3; MS (ESI) 955 (M+H).

20

| Compound | R <sub>1</sub> | R <sub>2</sub> | Amino Acid       |
|----------|----------------|----------------|------------------|
| 16a      | H              | Et             | Gly              |
| 16b      | H              | Bu             | Gly              |
| 16c      | Me             | Et             | Ala              |
| 16d      | Me             | Bu             | Ala              |
| 16e      | Et             | Et             | Aba <sup>1</sup> |
| 16f      | Et             | Bu             | Aba <sup>1</sup> |
| 16g      | iBu            | Et             | Leu              |
| 16h      | iBu            | Bu             | Leu              |
| 16i      | Bn             | Et             | Phe              |
| 16j      | Bn             | Bu             | Phe              |

<sup>1</sup> Aba, 2-aminobutyric acid

### Example 14

Diazo ketone 17: To a solution of N-tert-Butoxycarbonyl-p-bromo-L-phenylalanine (9.9 g, 25 28.8 mmol, Synthetech) in dry THF (55 mL) at -25-30°C (external bath temperature) was added isobutylchloroformate (3.74 mL, 28.8 mmol) followed by the slow addition of N-

methymorpholine (3.16 mL, 28.8 mmol). The mixture was stirred for 25 min, filtered while cold, and the filter cake was rinsed with cold (0°C) THF (50 mL). The filtrate was cooled to -25°C and diazomethane (~50 mmol, generated from 15 g diazald according to Aldrichimica Acta 1983, 16, 3) in ether (~150 mL) was poured into the mixed anhydride solution. The reaction was stirred for 15 min and was then placed in an icebath at 0°C, allowing the bath to warm to room temperature while stirring overnight for 15 h. The solvent was evaporated under reduced pressure and the residue was suspended in ether, washed with water, saturated NaHCO<sub>3</sub>, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated to a pale yellow solid. The crude solid was slurried in hexane, filtered, and dried to afford diazo ketone 17 (9.73 g, 90%) which was used directly in the next step.

#### Example 15

Chloroketone 18: To a solution of diazoketone 17 (9.73 g, 26 mmol) in ether (500 mL) at 0°C was added 4M HCl in dioxane (6.6 mL, 26 mmol). The solution was stirred for 1 h at 0°C and 4M HCl in dioxane (1 mL) was added. After 1h, the reaction solvent was evaporated under reduced pressure to afford the chloroketone 18 (9.79 g, 98%) as a white solid.

#### Example 16

Chloroalcohol 19: A solution of chloroketone 18 (9.79g, 26 mmol) in THF (180 mL) and water (16 mL) was cooled to 0°C (internal temperature). Solid NaBH<sub>4</sub> (2.5 g, 66 mmol) was added in several portions over a period of 15 min while maintaining the internal temperature below 5°C. The mixture was stirred for 45 min and saturated KHSO<sub>4</sub> was slowly added until the pH<3. The mixture was partitioned between EtOAc and water. The aqueous phase was extracted with EtOAc and the combined organic extracts were washed with brine, dried (MgSO<sub>4</sub>) filtered and evaporated under reduced pressure. The residue was dissolved in EtOAc, and was passed through a short column of silica gel, and the solvent was evaporated. The solid residue was recrystallized from EtOAc/hexane to afford the chloroalcohol 19 (3.84g) as a white solid.

#### Example 17

Epoxide 21: A partial suspension of chloroalcohol 19 (1.16g, 3.1 mmol) in EtOH (50 mL) was treated with K<sub>2</sub>CO<sub>3</sub> (2g, 14.5 mmol) and the mixture was stirred for 4 h at room

temperature. The reaction mixture was diluted with EtOAc, filtered, and the solvents were evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl, and the organic phase was dried (MgSO<sub>4</sub>), filtered, and evaporated under reduced pressure to afford epoxide 21 (1.05g, 92%) as a white crystalline solid.

5

#### Example 18

Sulfonamide 22: To a solution of epoxide 21 (1.05g, 3.1 mmol) in 2-propanol (40 mL) was added isobutylamine (6 mL, 61 mmol) and the solution was refluxed for 30 min. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and cooled to 0°C. Triethylamine (642 µL, 4.6 mmol) was added followed by the addition of (634 mg, 3.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the solution was stirred for 2h at 0°C at which time the reaction solution was treated with additional triethylamine (1.5 mmol) and 4-methoxybenzenesulfonyl chloride (0.31 mmol). After 1.5 h, the reaction solution was evaporated under reduced pressure. The residue was partitioned between EtOAc and cold 15 1M H<sub>3</sub>PO<sub>4</sub>. The organic phase was washed with saturated NaHCO<sub>3</sub>, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and the solvent was evaporated under reduced pressure. The crude product was purified on silica gel (15/1 - CH<sub>2</sub>Cl<sub>2</sub>/EtOAc) to afford 1.67g of a solid which was recrystallized from EtOAc/hexane to give sulfonamide 22 (1.54g, 86%) as a white crystalline solid.

20

#### Example 19

Silyl ether 23: To a solution of the sulfonamide 22 (1.53g, 2.6 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (12 mL) at 0°C was added N,N-diisopropylethylamine (0.68 mL, 3.9 mmol) followed by tert-butyltrimethylsilyl trifluoromethanesulfonate (0.75 mL, 3.3 mmol). The reaction solution 25 was stirred for 1 h at 0°C and was warmed to room temperature, stirring for 17 h. Additional N,N-diisopropylethylamine (3.9 mmol) and tert-butyltrimethylsilyl trifluoromethanesulfonate (1.6 mmol) was added, stirred for 2.5h, then heated to reflux for 3h and stirred at room temperature for 12 h. The reaction mixture was partitioned between EtOAc and cold 1M H<sub>3</sub>PO<sub>4</sub>. The organic phase was washed with saturated NaHCO<sub>3</sub>, saturated NaCl, and was 30 dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was purified on silica gel (2/1 - hexane/ether) to afford silyl ether 23 (780 mg, 43%) as an oil.

#### Example 20

Phosphonate 24: A solution of 23 (260 mg, 0.37 mmol), triethylamine (0.52 mL, 3.7 mmol), and diethylphosphite (0.24 mmol, 1.85 mmol) in toluene (2 mL) was purged with argon and to the solution was added  $(\text{Ph}_3\text{P})_4\text{Pd}$  (43 mg, 10 mol%). The reaction mixture was heated at 110°C (bath temperature) for 6 h, and was then allowed to stir at room temperature for 12h.

- 5 The solvent was evaporated under reduced pressure and the residue was partitioned between ether and water. The aqueous phase was extracted with ether and the combined organic extracts were washed with saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered, and the solvent was evaporated under reduced pressure. The residue was purified by chromatography on silica gel (2/1 - ethyl acetate/hexane) to afford diethylphosphonate 24 (153 mg, 55%).

10

#### Example 21

Phosphonic acid 26: To a solution of 24 (143 mg) in MeOH (5 mL) was added 4N HCl (2 mL). The solution was stirred at room temperature for 9h and was evaporated under reduced pressure. The residue was triturated with ether and the solid was collected by filtration to

- 15 provide hydrochloride salt 25 (100 mg, 92%) as a white powder. To a solution of X (47 mg, 0.87 mmol) in  $\text{CH}_3\text{CN}$  (1 mL) at 0°C was added TMSBr (130  $\mu\text{L}$ , 0.97 mmol). The reaction was warmed to room temperature and stirred for 6.5h at which time TMSBr (0.87 mmol) was added and stirring was continued for 16h. The solution was cooled to 0°C and was quenched with several drops of ice-cold water. The solvents were evaporated under reduced pressure
- 20 and the residue was dissolved in several milliliters of MeOH and treated with propylene oxide (2 mL). The mixture was heated to gentle boiling and evaporated. The residue was triturated with acetone and the solid was collected by filtration to give phosphonic acid 26 (32 mg, 76%) as a white solid.

#### Example 22

Phosphonate 27: To a suspension of 26 (32 mg, 0.66 mmol) in  $\text{CH}_3\text{CN}$  (1 mL) was added bis(trimethylsilyl)acetamide (100  $\mu\text{L}$ , 0.40 mmol) and the solution was stirred for 30 min at room temperature. The solvent was evaporated under reduced pressure and the residue was dissolved in  $\text{CH}_3\text{CN}$  (1 mL). To this solution was added (3R, 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (20 mg, 0.069 mmol, prepared according to Ghosh et al. J. Med. Chem. 1996, 39, 3278.), N,N-diisopropylethylamine (35  $\mu\text{L}$ , 0.20 mmol), and N,N-dimethylaminopyridine (catalytic amount). The solution was stirred for 22h at room temperature, diluted with water (0.5 mL) and was stirred with IR 120 ion exchange resin (325

mg, H<sup>+</sup> form) until the pH was <2. The resin was removed by filtration, washed with methanol and the filtrate was concentrated under reduced pressure. The residue was dissolved in water, treated with solid NaHCO<sub>3</sub> until pH=8 and was evaporated to dryness. The residue was dissolved in water and was purified on C18 reverse phase chromatography eluting with water followed by 5%, 10% and 20% MeOH in water to give the disodium salt 27 (24 mg) as a pale yellow solid: <sup>1</sup>H NMR (D<sub>2</sub>O) δ 7.72 (d, 2H), 7.52 (dd, 2H), 7.13 (dd, 2H), 7.05 (d, 2H), 5.58 (d, 1H), 4.87 (m, 1H), 3.86-3.53 (m overlapping s, 10H), 3.22 (dd, 1H), 3.12-2.85 (6H), 2.44 (m, 1H), 1.83 (m, 1H), 1.61 (m, 1H), 1.12 (dd, 1H), 0.77 (m, 6H); <sup>31</sup>P NMR (D<sub>2</sub>O) δ 11.23; MS (ESI) 641 (M-H).

#### Example 23

Diethylphosphonate 28: To a solution of 25 (16 mg, 0.028 mmol) in CH<sub>3</sub>CN (0.5 mL) was added (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (9 mg, 0.031 mmol), N,N-diisopropylethylamine (20 μL, 0.11 mmol), and N,N-dimethylaminopyridine (catalytic amount). The solution was stirred at room temperature for 48 h and was then concentrated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaHCO<sub>3</sub>, saturated NaCl, and was dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (2.5-5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>). The residue obtained was further purified by preparative layer chromatography (5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) followed by column chromatography on silica gel (10% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to afford diethylphosphonate 28 (7 mg) as a foam: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72-7.66 (m, 4H), 7.32-7.28 (2H), 6.96 (d, 2H), 5.60 (d, 1H), 4.97 (m, 2H), 4.18-4.01 (m, 4H), 3.94-3.60 (m overlapping s, 10H), 3.15-2.72 (m, 7H), 1.78 (m, 1H), 1.61 (m+H<sub>2</sub>O, ~3H), 1.28 (t; 6H), 0.86 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 18.6; MS (ESI) 699 (M+H).

#### Prospective Example 24

Diphenyl phosphonate 14 is treated with aqueous sodium hydroxide to provide monophenyl phosphonate 29 according to the method found in J. Med. Chem. 1994, 37, 1857.

Monophenyl phosphonate 29 is then converted to the monoamidate 30 by reaction with an amino acid ester in the presence of Ph<sub>3</sub> and 2,2'-dipyridyl disulfide as described in the synthesis of bisamidate 16f. Alternatively, monoamidate 30 is prepared by treating 29 with an



amino acid ester and DCC. Coupling conditions of this type are found in Bull. Chem. Soc. Jpn. 1988, 61, 4491.

#### Example 25

- 5 Diazo ketone 1: To a solution of N-tert-Butoxycarbonyl-O-benzyl-L-tyrosine (25 g, 67 mmol, Fluka) in dry THF (150 mL) at -25-30°C (external bath temperature) was added isobutylchloroformate (8.9 mL, 69 mmol) followed by the slow addition of N-methylmorpholine (37.5 mL, 69 mmol). The mixture was stirred for 40 min, and diazomethane (170 mmol, generated from 25 g 1-methyl-3-nitro-1-nitroso-guanidine
- 10 according to Aldrichimica Acta 1983, 16, 3) in ether (400 mL) was poured into the mixed anhydride solution. The reaction was stirred for 15 min allowing the bath to warm to room temperature while stirring overnight for 4 h. The mixture was bubbled with N<sub>2</sub> for 30 min., washed with water, saturated NaHCO<sub>3</sub>, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated to a pale yellow solid. The crude solid was slurried in hexane, filtered, and dried
- 15 to afford the diazo ketone (26.8 g, 99%) which was used directly in the next step.

#### Example 26

- Chloroketone 2: To a suspension of diazoketone 1 (26.8 g, 67 mmol) in ether/THF (750 mL, 3/2) at 0°C was added 4M HCl in dioxane (16.9 mL, 67 mmol). The solution was stirred at
- 20 0°C for 2 hr. The reaction solvent was evaporated under reduced pressure to give the chloroketone (27.7 g, 97%) as a solid.

#### Example 27

- Chloroalcohol 3: To a solution of chloroketone 2 (127.1 g, 67 mmol) in THF (350 mL) was
- 25 added water (40 mL) and the solution was cooled to 3-4°C (internal temperature). NaBH<sub>4</sub> (6.3 g, 168 mmol) was added in portions. The mixture was stirred for 1h at 0°C and the solvents were removed. The mixture was diluted with ethyl acetate and saturated KHSO<sub>4</sub> was slowly added until the pH<4 followed by saturated NaCl. The organic phase was washed with saturated NaCl, dried (MgSO<sub>4</sub>) filtered and evaporated under reduced pressure. The
- 30 crude product consisted of a 70:30 mixture of diastereomers by HPLC analysis (mobile phase, 77:25-CH<sub>3</sub>CN:H<sub>2</sub>O; flow rate: 1 mL/min; detection: 254 nm; sample volume: 20 µL; column: 5µ C18, 4.6X250 mm, Varian; retention times: major diastereomer 3, 5.4 min, minor

diastereomer 4, 6.1 min). The residue was recrystallized from EtOAc/hexane twice to afford the chloro alcohol 3 (12.2g, >96% diastereomeric purity by HPLC analysis) as a white solid.

#### Example 28

- 5 Epoxide 5: To a solution of chloroalcohol 3 (12.17 g, 130 mmol) in EtOH (300 mL) was added KOH/EtOH solution (0.71N, 51 mL, 36 mmol). The mixture was stirred for at room temperature for 1.5h. The reaction mixture was evaporated under reduced pressure. The residue was partitioned between EtOAc and water and the organic phase was washed with saturated NH<sub>4</sub>Cl, dried (MgSO<sub>4</sub>), filtered, and evaporated under reduced pressure to afford
- 10 the epoxide (10.8 g, 97%) as a white solid.

#### Example 29

- Sulfonamide 6: To a suspension of epoxide 5 (10.8 g, 30 mmol) in 2-propanol (100 mL) was added isobutylamine (129.8 mL, 300 mmol) and the solution was refluxed for 1 hr. The
- 15 solution was evaporated under reduced pressure to give a crude solid. The solid (42 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) and cooled to 0°C. Triethylamine (11.7 mL, 84 mmol) was added followed by the addition of 4-methoxybenzenesulfonyl chloride (8.68 g, 42 mmol) and the solution was stirred for 40 min at 0°C, warmed to room temperature and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>.
- 20 The organic phase was washed with saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexane to give the sulfonamide (23.4 g, 91%) as a small white needles: mp 122-124°C (uncorrected).

#### Example 30

- 25 Carbamate 7: A solution of sulfonamide 6 (6.29 mg, 10.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was treated with trifluoroacetic acid (10 mL). The solution was stirred for 3 hr. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase were washed with 0.5 N NaOH (2x), water (2x) and saturated NaCl, dried (MgSO<sub>4</sub>), filtered, and evaporated under reduced pressure. The residue was
- 30 dissolved in CH<sub>3</sub>CN (60 mL), cooled to 0°C and was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (298.5 g, 10 mmol, prepared according to Ghosh et al. J. Med. Chem. 1996, 39, 3278.) and N,N-dimethylaminopyridine (2.4 g, 20 mmol). After stirring for 1h at 0°C, the reaction solvent was evaporated under

reduced pressure and the residue was partitioned between EtOAc and 5% citric acid. The organic phase was washed twice with 1%  $K_2CO_3$ , and then was washed with saturated NaCl, dried ( $MgSO_4$ ), filtered, and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (1/1 - EtOAc/hexane) affording the carbamate (5.4 g, 83%) as a solid: mp 128-129°C (MeOH, uncorrected).

#### Example 31

Phenol 8: A solution of carbamate 7 (5.4 g, 8.0 mmol) in EtOH (260 mL) and EtOAc (130 mL) was treated with 10% Pd/C (540 mg) and was stirred under  $H_2$  atmosphere (balloon) for 3h. The reaction solution stirred with celite for 10 min, and passed through a pad of celite. The filtrate was evaporated under reduced pressure to afford the phenol as a solid (4.9 g) that contained residual solvent: mp 131-134°C (EtOAc/hexane, uncorrected).

#### Example 32

Dibenzylphosphonate 10: To a solution of dibenzylhydroxymethyl phosphonate (3.1 g, 10.6 mmol) in  $CH_2Cl_2$  (30 mL) was treated with 2,6-lutidine (1.8 mL, 15.6 mmol) and the reaction flask was cooled to -50°C (external temperature). Trifluoromethanesulfonic anhydride (2.11 mL, 12.6 mmol) was added and the reaction mixture was stirred for 15 min and then the cooling bath was allowed to warm to 0°C over 45 min. The reaction mixture was partitioned between ether and ice-cold water. The organic phase was washed with cold 1M  $H_3PO_4$ , saturated NaCl, dried ( $MgSO_4$ ), filtered and evaporated under reduced pressure to afford triflate 9 (3.6 g, 80%) as an oil which was used directly without any further purification. To a solution of phenol 8 (3.61 g, 6.3 mmol) in THF (90 mL) was added  $Cs_2CO_3$  (4.1 g, 12.6 mmol) and triflate 9 (4.1 g, 9.5 mmol) in THF (10 mL). After stirring the reaction mixture for 30 min at room temperature additional  $Cs_2CO_3$  (6.96 g, 3 mmol) and triflate (1.26 g, 3 mmol) were added and the mixture was stirred for 3.5h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried ( $MgSO_4$ ), filtered and evaporated under reduced pressure. The crude product was chromatographed on silica gel eluting (5% 2-propanol/ $CH_2Cl_2$ ) to give the dibenzylphosphonate as an oil that solidified upon standing. The solid was dissolved in EtOAc, ether was added, and the solid was precipitated at room temperature overnight. After cooling to 0°C the solid was filtered and washed with cold ether to afford the dibenzylphosphonate (3.43 g, 64%) as a white solid:  $^1H$  NMR ( $CDCl_3$ )  $\delta$  7.66

(d, 2H), 7.31 (s, 10H), 7.08 (d, 2H), 6.94 (d, 2H), 6.76 (d, 2H), 5.59 (d, 1H), 5.15-4.89 (m, 6H), 4.15 (d, 2H), 3.94-3.62 (m, 10H), 3.13-2.69 (m, 7H), 1.78 (m, 1H), 1.70-1.44 (m, 2H), 0.89-0.82 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  18.7; MS (ESI) 853 (M+H).

5 Example 33

Phosphonic acid 11: A solution of dibenzylphosphonate 10 (3.43 g) was dissolved in EtOH/EtOAc (150 mL/50 mL), treated with 10% Pd/C (350 mg) and was stirred under  $\text{H}_2$  atmosphere (balloon) for 3 h. The reaction mixture was stirred with celite, and the catalyst was removed by filtration through celite. The filtrate was evaporated under reduced pressure

10 and the residue was dissolved in MeOH and filtered with a 0.45  $\mu\text{m}$  filter. After evaporation of the filtrate, the residue was triturated with ether and the solid was collected by filtration to afford the phosphonic acid (2.6 g, 94%) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.77 (d, 2H), 7.19 (d, 2H), 7.09 (d, 2H), 6.92 (d, 2H), 5.60 (d, 1H), 4.95 (m, 1H), 4.17 (d, 2H), 3.94 (m, 1H), 3.89 (s, 3H), 3.85-3.68 (m, 5H), 3.42 (dd, 1H), 3.16-3.06 (m, 2H), 2.96-2.84 (m, 3H),  
15 2.50 (m, 1H), 2.02 (m, 1H), 1.58 (m, 1H), 1.40 (dd, 1H), 0.94 (d, 3H), 0.89 (d, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.2; MS (ESI) 671 (M-H).

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Example Section B

There is no Section B in this application.

Example Section CExample 1

Diphenyl phosphonate 31: To a solution of phosphonic acid 30 (11 g, 16.4 mmol) and phenol  
5 (11 g, 117 mmol) in pyridine (100 mL) was added 1, 3-dicyclohexylcarbodiimide (13.5 g,  
65.5 mmol). The solution was stirred at room temperature for 5 min and then at 70°C for 2h.  
The reaction mixture was cooled to room temperature, diluted with ethyl acetate (100 mL)  
and filtered. The filtrate was evaporated under reduced pressure to remove pyridine. The  
residue was dissolved in ethyl acetate (250 mL) and acidified to pH = 4 by addition of HCl  
10 (0.5 N) at 0°C. The mixture was stirred at 0°C for 0.5 h, filtered and the organic phase was  
separated and washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced  
pressure. The residue was purified on silica gel to give diphenyl phosphonate 31 (9 g, 67%)  
as a solid. <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 12.5.

Example 2

Monophenyl phosphonate 32: To a solution of diphenylphosphonate 31 (9.0 g, 10.9 mmol) in  
acetonitrile (400 mL) was added NaOH (1N, 27 mL) at 0°C. The reaction mixture was stirred at  
0°C for 1 h, and then treated with Dowex (50WX8-200, 12 g). The mixture was stirred for 0.5 h  
at 0°C, and then filtered. The filtrate was concentrated under reduced pressure and co-  
20 evaporated with toluene. The residue was dissolved in ethyl acetate and hexane was added to  
precipitate out the monophenyl phosphonate 32 (8.1 g, 100%). <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 18.3.

Example 3

Monoamidate 33a (R<sub>1</sub> = Me, R<sub>2</sub> = n-Bu): To a flask charged with monophenyl phosphonate  
25 32 (4.0 g, 5.35 mmol), was added L-alanine n-butyl ester hydrochloride (4.0 g, 22 mmol), 1,  
3-dicyclohexylcarbodiimide (6.6 g, 32 mmol), and finally pyridine (30 mL) under nitrogen.  
The resultant mixture was stirred at 60 – 70°C for 1 h, then cooled to room temperature and  
diluted with ethyl acetate. The mixture was filtered and the filtrate was concentrated under  
reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2 N) and the  
30 organic layer was separated. The ethyl acetate phase was washed with water, saturated  
NaHCO<sub>3</sub>, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue  
was purified on silica gel (pre-treated with 10% MeOH / CH<sub>3</sub>CO<sub>2</sub>Et, eluting with 40%  
CH<sub>2</sub>Cl<sub>2</sub> / CH<sub>3</sub>CO<sub>2</sub>Et and CH<sub>3</sub>CO<sub>2</sub>Et) to give two isomers of 33a in a total yield of 51%.

- Isomer A (1.1 g):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (m, 9H), 1.3 (m, 2H), 1.35 (d,  $J = 7$  Hz, 3H), 1.55 (m, 2H), 1.55-1.7 (m, 2H), 1.8 (m, 1H), 2.7-3.2 (m, 7H), 3.65-4.1 (m, 9H), 3.85 (s, 3H), 4.2 (m, 1H), 4.3 (d,  $J = 9.6$  Hz, 2H), 5.0 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 6.85 (d,  $J = 8.7$  Hz, 2H), 7.0 (d,  $J = 8.7$  Hz, 2H), 7.1-7.3 (m, 7H), 7.7 (d,  $J = 8.7$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.5.
- 5 Isomer B (1.3 g)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.88 (m, 9H), 1.3 (m, 2H), 1.35 (d,  $J = 7$  Hz, 3H), 1.55 (m, 2H), 1.55-1.7 (m, 2H), 1.8 (m, 1H), 2.7-3.2 (m, 7H), 3.65-4.1 (m, 9H), 3.85 (s, 3H), 4.2-4.35 (m, 3H), 5.0 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 6.85 (d,  $J = 8.7$  Hz, 2H), 7.0 (d,  $J = 8.7$  Hz, 2H), 7.1-7.3 (m, 7H), 7.7 (d,  $J = 8.7$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.4.

10 Example 4

- Monoamidate **33b** ( $\text{R}_1 = \text{Me}$ ,  $\text{R}_2 = i\text{-Pr}$ ) was synthesized in the same manner as **33a** in 77% yield. Isomer A:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.9 (2d,  $J = 6.3$  Hz, 6H), 1.2 (d,  $J = 7$  Hz, 6H), 1.38 (d,  $J = 7$  Hz, 3H), 1.55-1.9 (m, 3H), 2.7-3.2 (m, 7H), 3.65-4.1 (m, 8H), 3.85 (s, 3H), 4.2 (m, 1H), 4.3 (d,  $J = 9.6$  Hz, 2H), 5.0 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 6.85 (d,  $J = 8.7$  Hz, 2H), 7.0 (d,  $J = 8.7$  Hz, 2H), 7.1-7.3 (m, 7H), 7.7 (d,  $J = 8.7$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4.
- 15 Isomer B:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.9 (2d,  $J = 6.3$  Hz, 6H), 1.2 (d,  $J = 7$  Hz, 6H), 1.38 (d,  $J = 7$  Hz, 3H), 1.55-1.9 (m, 3H), 2.7-3.2 (m, 7H), 3.65-4.1 (m, 8H), 3.85 (s, 3H), 4.2 (m, 1H), 4.3 (d,  $J = 9.6$  Hz, 2H), 5.0 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 6.85 (d,  $J = 8.7$  Hz, 2H), 7.0 (d,  $J = 8.7$  Hz, 2H), 7.1-7.3 (m, 7H), 7.7 (d,  $J = 8.7$  Hz, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.5.

### Example Section D

#### Example 1

Cyclic Anhydride 1 (6.57 g, 51.3 mmol) was treated according to the procedure of Brown et al., J. Amer. Chem. Soc. 1955, 77, 1089 -1091 to afford amino alcohol 3 (2.00g, 33%). *for*  
5 *intermediate 2* :  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  2.40 (s, 2H), 1.20 (s, 6H).

#### Example 2

Amino alcohol 3 (2.0 g, 17 mmol) was stirred in 30 mL 1:1 THF: water. Sodium  
10 Bicarbonate (7.2 g, 86 mmol) was added, followed by Boc Anhydride (4.1 g, 19 mmol). The reaction was stirred for 1 hour, at which time TLC in 5% methanol/DCM with ninhydrin stain showed completion. The reaction was partitioned between water and ethyl acetate. The organic layer was dried and concentrated, and the resulting mixture was chromatographed on silica in 1:1 hexane: ethyl acetate to afford two fractions, "upper" and "lower" each having  
15 the correct mass. By NMR the correct product 4 was "lower" (0.56 g, 14%)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  3.7 (t, 2H), 3.0 (d, 2H), 1.45 (t, 2H) 1.4 (s, 9H), 0.85 (s, 6H), MS (ESI): 240 (M + 23).

#### Example 3

20 Sodium Hydride (60% emulsion in oil) was added to a solution of the alcohol 4 (1.1g, 5.2 mmol) in dry DMF in a 3-neck flask under dry nitrogen. Shortly afterward triflate 35 (2.4 g, 5.7 mmol) was added with stirring for 1.5 hrs. Mass spectrometry showed the presence of the starting material (240, M+23), thus 100 mg more 60% sodium hydride emulsion as well as ~1 g more triflate were added with an additional hour of stirring. The  
25 reaction was quenched by the addition of saturated  $\text{NaHCO}_3$  then partitioned between ethyl acetate and water. The organic layer was dried with brine and  $\text{MgSO}_4$  and eluted on silica with 1:1 hexane:ethyl acetate to afford 5 (0.445 g, 15%). NMR showed some contamination with alcohol 4 starting material.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.28 (s, 10H), 5.00 (m, 4H), 3.70 (t, 2H), 2.94, (d, 2H), 1.44 (t, 2H), 1.40 (s, 9H), 0.83 (s, 6H) MS (ESI): 514 (M+23).

30

#### Example 4

Phosphonate ester 5 (0.445 g, 0.906 mmol) was stirred with with 20% TFA in DCM. (5 mL) TLC showed completion in 1 hr time. The reaction was azeotroped with toluene then run on



a silica gel column with 10% methanol in DCM. Subsequently, the product was dissolved in ethyl acetate and shaken with saturated sodium bicarbonate: water (1:1), dried with brine and magnesium sulfate to afford the free amine 6 (30mg, 8.5%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.30 (s, 10H), 5.00 (m, 4H), 3.67 (d, 2H), 3.47, (t, 2H), 2.4-2.6 (brs) 1.45 (t, 2H), 0.82 (s, 6H), MS (ESI): 393 (M+1).

#### Example 5

Amine 6 (30 mg, 0.08 mmol) and epoxide 7 (21 mg, 0.08 mmol) were dissolved in 2 mL IprOH and heated to reflux for 1 hr then monitored by TLC in 10% MeOH/DCM. Added  
10 ~20 mg more epoxide 7 and continued reflux for 1 hr. Cool to room temperature, dilute with ethyl acetate, shake with water and brine, dry with magnesium sulfate. Silica gel chromatography using first 5% then 10% MeOH in EtOAc yielded amine 8 (18 mg, 36%).  
<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.30 (s, 10H), 7.20-7.14 (m, 5H), 5.25-4.91 (m, 4H), 3.83, (m, 1H), 3.71 (d, 2H) 3.64 (m, 1H), 3.54 (t, 2H), 3.02-2.61 (m, 5H), 2.65-2.36 (dd, 2H) (t, 2H), 1.30 (s, 9H)  
15 0.93 (s, 9H) 0.83 (t, 2H) MS (ESI) 655 (M+1).

#### Example 6

Amine 8 (18 mg, 0.027 mmol) was dissolved in 1 mL DCM then acid chloride 9 (6 mg, 0.2 mmol) followed by triethylamine (0.004 mL, 0.029 mmol). The reaction was monitored by  
20 TLC. Upon completion the reaction was diluted with DCM shaken with 5% citric acid, saturated sodium bicarbonate, brine, and dried with MgSO<sub>4</sub>. Purification on silica (1:1 Hexane:EtOAc) afforded sulfonamide 10 (10.5 mg, 46%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.69 (d, 2H), 7.30 (s, 10H), 7.24-7.18 (m, 5H), 5.00 (m, 4H), 4.73, (d, 1H), 4.19 (s, 1H) 3.81 (m, 1H), 3.80 (s, 3H), 3.71 (d, 2H), 3.57 (t, 2H), 3.11-2.95 (m, 5H) 2.75 (m, 1H) 1.25 (s, 1H), 0.90 (s, 6H)  
25 MS (ESI) 847 (M+Na<sup>+</sup>).

#### Example 7

Sulfonamide 10 (10.5 mg, 0.013 mmol) was stirred at room temperature in 20% TFA/DCM. Once Boc deprotection was complete by TLC (1:1 Hexane:EtOAc) and MS, the  
30 reaction was azeotroped with toluene. The TFA salt of the amine was dissolved in acetonitrile (0.5 mg) and to this were added carbonate 11 (4.3 mg, 0.014 mmol) followed by DMAP (4.6 mg, 0.038 mg). Stir at room temp until TLC (1:1 Hexane:EtOAc) shows completion. Solvent was evaporated and the residue was redissolved in EtOAc then shaken

with saturated  $\text{NaHCO}_3$ . The organic layer was washed with water and brine, then dried with  $\text{MgSO}_4$ . Purification on silica with Hexane: EtOAc afforded compound 12 (7.1 mg, 50%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.75 (d, 2H) 7.24-7.35 (15H) 6.98 (d, 2H), 5.62 (d, 1H) 5.04 (m, 4H) 4.98 (m, 1H) 4.03 (m, 1H), 3.85 (s, 3H), 3.61-3.91 (9H), 3.23-3.04 (5H) 2.85 (m, 1H), 2.74 (m, 1H) 1.61 (d, 2H), 1.55 (m, 1H) 1.36 (m, 1H) 0.96 (d, 6H) MS (ESI): 903 (M+23).

#### Example 8

Compound 12 (6.1 mg, 0.007 mmol) was dissolved in 1 mL 3:1 EtOH:EtOAc. Palladium catalyst (10% on C, 1mg) was added and the mixture was purged three times to vacuum with 1 atmosphere hydrogen gas using a balloon. The reaction was stirred for 2 hrs, when MS and TLC showed completion. The reaction was filtered through Celite with EtOH washing and all solvent to was evaporated to afford final compound 13 (5mg, 100%).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.79 (d, 2H) 7.16-7.24 (5H) 7.09 (d, 2H) 5.58 (d, 1H) 4.92 (m, 1H) 3.97 (m, 1H), 3.92 (dd, 1H) 3.89 (s, 3H) 3.66-3.78 (8H) 3.40 (d, 1H), 3.37 (dd, 1H), 3.15 (m, 1H) 3.12 (dd, 1H) 2.96 (d, 1H), 2.87 (m, 1H), 2.74 (m, 1H) 2.53 (m, 1H) 1.70 (m, 2H), 1.53 (m, 1H) 1.32 (m, 1H) 1.04 (d, 6H) MS (ESI): 723 (M+23).

#### Example 9

Amino Alcohol 14 (2.67g, 25.9 mmol) was dissolved in THF with stirring and Boc Anhydride (6.78g, 31.1 mmol) was added. Heat and gas evolution ensued. TEA (3.97 mL, 28.5 mmol) was added and the reaction was stirred overnight. In the morning, the reaction was quenched by the addition of saturated  $\text{NaHCO}_3$ . The organic layer was separated out and shaken with water, dried with brine and  $\text{MgSO}_4$  to afford 15 which was used without further purification. (100% yield) (some contamination):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  3.76 (t, 1H) 3.20, (d, 2H), 2.97 (d, 2H), 1.44 (s, 9H), 0.85 (s, 6H).

#### Example 10

A solution of the alcohol 15 (500 mg, 2.45 mmol) in dry THF was cooled under dry  $\text{N}_2$  with stirring. To this was added n-butyl lithium (1.29 mL, 2.71 mmol) as a solution in hexane in a manner similar to that described in Tetrahedron. 1995, 51 #35, 9737-9746. Triflate 35 (1.15 g, 2.71 mmol) was added neat with a tared syringe. The reaction was stirred for four hours, then quenched with saturated  $\text{NaHCO}_3$ . The mixture was then partitioned between water and EtOAc. The organic layer was dried with brine and  $\text{MgSO}_4$ , then chromatographed on silica

in 1:1 Hexane:EtOAc to afford phosphonate 16 (445mg, 38%)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.37 (m, 10H), 5.09 (m, 4H), 3.73-3.75 (m, 2H), 3.24 (s, 2H), 3.02 (d, 2H), 1.43 (s, 9H), 0.86 (s, 6H).

#### Example 11

- 5 Phosphonate 16 (249 mg, 0.522 mmol) was stirred in 20% TFA/DCM for 1 hr. The reaction was then azeotroped with toluene. The residue was re-dissolved in EtOAc, then shaken with water: saturated  $\text{NaHCO}_3$  (1:1). The organic layer was dried with brine and  $\text{MgSO}_4$  and solvent was removed to afford amine 17 (143 mg, 73%)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.30 (s, 10H), 5.05-4.99 (m, 4H), 3.73 (d, 2H), 3.23 (s, 2H), 2.46 (brs, 2H), 0.80 (s, 6H)  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ):
- 10  $\delta$  23.77 (s).

#### Example 12

- Amine 17 (143 mg, 0.379 mmol) and epoxide 7 (95 mg, 0.360 mmol) were dissolved in 3 mL  $\text{iPrOH}$  and heated to  $85^\circ\text{C}$  for 1 hr. The reaction was cooled to room temperature overnight
- 15 then heated to  $85^\circ\text{C}$  for 1 hr more in the morning. The reaction was then diluted with EtOAc, shaken with water, dried with brine  $\text{MgSO}_4$  and concentrated. The residue was eluted on silica in a gradient from 5% to 10% MeOH in DCM to afford compound 18 (33 mg, 14%).

#### Example 13

- 20 Mix compound 18 (33 mg, 0.051 mmol) and chlorosulfonyl compound 9 (11 mg, 0.054 mmol) in 2 mL DCM then add TEA (0.0075 mL, 0.054 mmol), stir for 5 hrs. TLC in 1:1 EtOAc: hexane shows reaction not complete. Place in freezer overnight. In the morning, take out of freezer, stir for 2 hrs, TLC shows completion. Workup done with 5% citric acid, saturated  $\text{NaHCO}_3$ , then dry with brine and  $\text{MgSO}_4$ . The reaction mixture was concentrated
- 25 and chromatographed on a Monster Pipette column in 1:1 hexane: EtOAc then 7:3 hexane: EtOAc to avail compound 19 (28 mg, 67%)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.37 (d, 2H), 7.20 (m, 15H), 6.90 (d, 2H), 5.07-4.93 (m, 4H), 4.16 (brs, 1H), 3.80 (s, 3H), 3.75-3.37 (m, 4H), 3.36 (d, 1H), 3.20-2.93 (m, 6H), 2.80-2.75 (dd, 1H).

#### 30 Example 14

Compound 19 (28 mg, 0.35 mmol) was stirred in 4 mL DCM with addition of 1 mL TFA. Stir for 45 minutes, at which time complete deprotection was noted by TLC as well as MS. Azeotrope with toluene. The residue was dissolved in 1 mL  $\text{CH}_3\text{CN}$ , cooled to  $0^\circ\text{C}$ . Bis-

Furan *para*-Nitro phenol carbonate 11 (12 mg, 0.038 mmol), dimethyl amino pyridine (~ 1 mg, 0.008 mmol) and diisopropylethylamine (0.018 mL, 0.103 mmol) were added. The mixture was stirred and allowed to come to room temperature and stirred until TLC in 1:1 hexane:EtOAc showed completion. The reaction mixture was concentrated and the residue was partitioned between saturated NaHCO<sub>3</sub> and EtOAc. The organic layer was dried with brine and MgSO<sub>4</sub>, then chromatographed on silica with hexane:EtOAc to afford compound 20 (20 mg, 67%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.76 (d, 2H), 7.34–7.16 (m, 15 H), 7.07 (d, 2H), 5.56 (d, 1H), 5.09 (m, 4H), 4.87 (m, 1H), 4.01 (m, 1H), 3.91 (m, 2H), 3.87 (s, 3H), 3.86 (m, 1H), 3.69 (m, 1H), 3.67 (m, 1H) 3.60 (d, 2H) 3.28 (m, 1H) 3.25 (d, 2H), 3.32 (d, 1H), 3.13 (m, 1H), 3.02 (m, 1H) 2.85 (d, 1H), 2.83 (m, 1H) 2.52 (m, 1H) 1.47 (m, 1H), 1.31 (m, 1H) 0.98 (s, 3H), 0.95 (s, 3H).

#### Example 15

Compound 20 (7 mg, 0.008 mmol) was treated in a manner identical to example 8 to afford compound 21 (5 mg, 90%) <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.80 (d, 2H), 7.25–7.16 (m, 5H), 7.09 (d, 2H), 5.58 (d, 1H), 4.92 (m, 1H), 3.99 (m, 1H), 3.92 (m, 1H), 3.88 (s, 3H), 3.86 (m, 1H), 3.77 (m, 1H), 3.75 (m, 1H), 3.73 (m, 1H), 3.71 (m, 1H) 3.71 (m, 1H), 3.68 (m, 1H), 3.57 (d, 1H), 3.41 (d, 1H), 3.36 (m, 1H), 3.29 (d, 1H), 3.25 (d, 2H), 3.18 (m, 1H), 3.12 (m, 1H), 3.01 (d, 1H) 2.86 (m, 1H), 2.53 (m, 1H) 1.50 (m, 1H), 1.33 (m, 1H), 1.02 (s, 3H), 0.99 (s, 3H).

#### Example 16

Compound 15 (1.86 g, 9.20 mmol) was treated with triflate 22 in a manner identical to example 10 to afford compound 23 (0.71 g, 21.8%) <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.21 (brs, 1H) 4.16–4.07 (m, 4H), 3.71–3.69 (d, 2H), 3.24 (s, 2H), 1.43 (s, 9H), 1.34–1.28 (m, 6H) 0.86 (s, 6H).

#### Example 17

Compound 23 (151 mg, 0.427 mmol) was dissolved in 10 mL DCM and 1.0 mL TFA was added. The reaction was stirred until completion. The reaction was azeotroped with toluene and the residue was then dissolved in THF and treated with basic Dowex resin beads. Afterwards, the beads were filtered away and solvent was removed to avail compound 24 (100 mg, 92%) <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.15–4.05 (m, 4H), 3.72–3.69 (d, 2H), 3.27 (s, 2H), 1.30–1.26 (m, 6H) 0.81 (s, 6H).

Example 18

Compound 24 (100 mg, 0.395 mmol) was treated in a manner identical to example 12 to afford compound 25 (123 mg, 60%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.26–7.13 (m, 5H), 4.48–4.83 (d, 1H) 4.17–4.06 (m, 4H), 3.75 (d, 2H) 3.56 (brs, 1H), 3.33 (s, 2H), 2.93–2.69 (m, 4H), 2.44–2.55 (dd, 2H) 1.32 (m, 6H), 0.916 (s, 6H).

Example 19

Compound 25 (88 mg, 0.171 mmol) was treated in a manner identical to example 13 to afford compound 26 (65 mg, 55%)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.26–7.13 (m, 5H), 4.48–4.83 (d, 1H) 4.17–4.06 (m, 4H), 3.75 (d, 2H) 3.56 (brs, 1H), 3.33 (s, 2H), 2.93–2.69 (m, 4H), 2.44–2.55 (dd, 2H) 1.32 (m, 6H), 0.916 (s, 6H).

Example 20

Compound 26 (65 mg, 0.171 mmol) was treated in a manner identical to example 14 to afford compound 27 (49 mg, 70%)  $^1\text{H}$  NMR: ( $\text{CDCl}_3$ ):  $\delta$  7.75 (d, 2H), 7.25–7.24 (m, 4 H), 7.18 (m, 1H) 6.99 (d, 2H), 5.63 (d, 1H), 5.01 (m, 1H), 4.16 (m, 4H), 3.94 (m, 1H), 3.88 (m, 1H), 3.88 (s, 3H), 3.84 (m, 1H), 3.81 (m, 1H), 3.74 (m, 2H), ), 3.70 (m, 1H), 3.69 (m, 1H) 3.43 (m, 1H), 3.24 (m, 1H), 3.22 (m, 2H) 3.21 (m, 2H) 3.12 (m, 1H), 3.02 (m, 1H) 2.86 (m, 1H), 2.72 (m, 1H), 1.54 (m, 1H), 1.38 (m, 1H) 1.35 (m, 6H) 1.00 (s, 3H), 0.96 (s, 3H).

Example 21

Boc protected amine 28 (103 mg, 0.153 mmol) was dissolved in DCM (5 mL). The stirred solution was cooled to 0°C.  $\text{BBr}_3$  as a 1.0 M solution in DCM (0.92 mL, 0.92 mmol) was added dropwise over 10 min, and the reaction was allowed to continue stirring at 0°C for 20 min. The reaction was warmed to room temperature and stirring was continued for 2 hours. The reaction was then cooled to 0°C and quenched by dropwise addition of MeOH (1 mL). The reaction mixture was evaporated and the residue suspended in methanol which was removed under reduced pressure. The procedure was repeated for EtOAc and finally toluene to afford free amine HBr salt 29 (107 mg, >100%) which was used without further purification.

Example 22

Amine HBr salt 29 (50 mg, 0.102 mmol) was suspended in 2 mL CH<sub>3</sub>CN with stirring then cooled to 0°C. DMAP (25 mg, 0.205 mmol) was added, followed by Carbonate 11. The reaction was stirred at 0°C for 1.5 hrs then allowed to warm to room temperature. The reaction was stirred overnight. A few drops Acetic acid were added to the reaction mixture, which was concentrated and re-diluted with ethyl acetate, shaken with 10% citric acid then saturated NaHCO<sub>3</sub>. The organic layer was dried with brine and MgSO<sub>4</sub> and eluted on silica to afford di-phenol 30 (16 mg, 28%) <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.61, (d, 2H), 7.01 (d, 2H), 6.87 (d, 2H), 6.62 (d, 2H), 5.55 (d, 1H), 4.93 (m, 1H), 3.92 (m, 2H), 3.79 (m, 5H), 3.35 (m, 1H), 3.07 (m, 2H), 2.88 (m, 3H), 2.41 (m, 1H), 2.00 (m, 1H), 1.54 (m, 1H), 1.31 (dd, 1H) 0.89-0.82 (dd, 6H).

Example 23

A solution of di-phenol 30 (100 mg, 0.177 mmol) was made in CH<sub>3</sub>CN that had been dried over K<sub>2</sub>CO<sub>3</sub>. To this, the triflate (0.084 mL, 0.23 mmol) was added, followed by Cs<sub>2</sub>CO<sub>3</sub> (173 mg, 0.531 mmol). The reaction was stirred for 1 hr. TLC (5% IprOH/DCM) showed 2 spots with no starting materials left. Solvent was evaporated and the residue was partitioned between EtOAc and water. The organic layer was washed with saturated NaHCO<sub>3</sub>, then dried with brine and MgSO<sub>4</sub>. The mixture was separated by column chromatography on silica with 3% IprOH in DCM. The upper spot 31 (90 mg, 46%) was confirmed to be the *bis* alkylation product. The lower spot required further purification on silica gel plates to afford a single *mono* alkylation product 32 (37 mg, 26%). The other possible alkylation product was not observed. NMR : <sup>1</sup>H NMR (CDCl<sub>3</sub>): *for 31*: δ 7.57 (d, 2H), 7.37 (m, 10H) 7.03 (d, 2H), 6.99 (d, 2H), 6.73 (d, 2H), 5.69 (d, 1H), 5.15-5.09 (m, 4H), 5.10 (m, 1H), 4.32 (d, 2H), 4.02 (d, 1H), 3.82 (m, 1H) 3.81 (m, 1H), 3.93-3.81 (m, 2H), 3.74 (d, 1H), 3.06 (m, 1H), 3.00 (m, 1H), 2.96 (m, 1H), 2.91 (m, 1H) 2.77 (m, 1H) 2.64 (m, 1H) 2.47 (m, 1H) 1.82 (m, 2H) 1.79 (m, 1H), 0.94-0.86 (dd, 6H) *for 32*: δ 7.68 (d, 2H), 7.33-7.35 (m, 20H), 7.11 (d, 2H), 6.96 (d, 2H), 6.80 (d, 2H), 5.26 (d, 1H), 5.11 (m, 8H), 5.00 (m, 1H) 4.23 (d, 2H), 4.19 (d, 2H), 3.93 (m, 1H), 3.82-3.83 (m, 3H), 3.68-3.69 (m, 2H) 3.12-2.75 (m, 7H), 1.82 (m, 1H), 1.62-1.52 (d, 2H), 0.89-0.86 (dd, 6H).

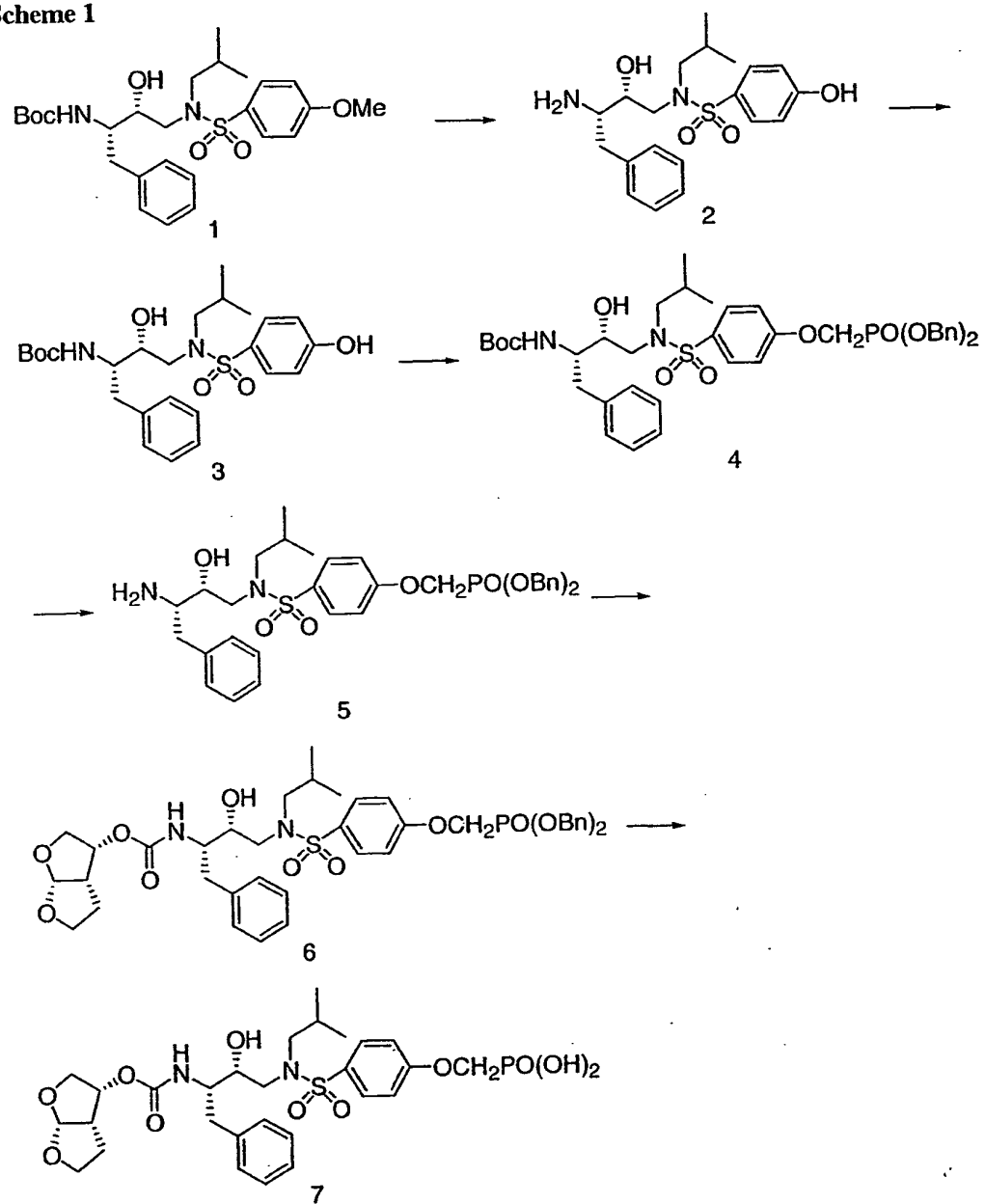
Example 24

Ref: J. Med. Chem. 1992, 35 10, 1681-1701

To a solution of phosphonate 32 (100 mg, 0.119 mmol) in dry dioxane was added  $\text{Cs}_2\text{CO}_3$  (233 mg, 0.715 mmol), followed by 2-(dimethylamino) ethyl chloride hydrochloride salt (69 mg, 0.48 mmol). The reaction was stirred at room temperature and monitored by TLC. When it was determined that starting material remained, additional  $\text{Cs}_2\text{CO}_3$  (233 mg, 0.715 mmol) as well as amine salt (69 mg, 0.48 mmol) were added and the reaction was stirred overnight at 60°C. In the morning when TLC showed completion the reaction was cooled to room temperature, filtered, and concentrated. The product amine 33 (40 mg, 37%) was purified on silica. Decomposition was noted as lower spots were seen to emerge with time using 15% MeOH in DCM on silica.

Example 25:

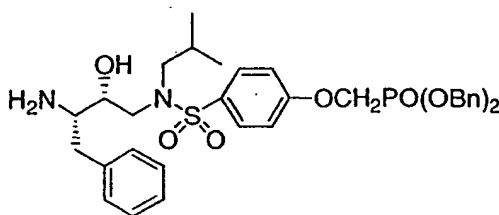
Amine 33 (19 mg, 0.021 mmol) was dissolved in 1.5 mL DCM. This solution was stirred in an icebath. Methane sulfonic acid (0.0015 mL, 0.023 mmol) was added and the reaction was stirred for 20 minutes. The reaction was warmed to room temperature and stirred for 1 hour. The product, amine mesylate salt 34 (20 mg, 95%) was precipitated out by addition of hexane.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.69 (d, 2H), 7.35 (m, 10H), 7.15 (m, 4H) 6.85 (m, 2H), 5.49 (d, 1H), 5.10 (m, 4H), 4.83 (m, 1H), 4.62 (d, 2H), 4.22 (m, 2H), 3.82 (m, 1H), 3.56 (m, 1H), 3.48 (m, 2H), 3.35 (m, 1H), 2.99 (m, 1H), 2.95 (m, 1H), 2.84 (s, 6H), 2.78 (m, 1H), 2.75 (m, 1H), 2.70 (m, 1H), 2.40 (m, 1H) 1.94 (m, 1H), 1.43 (m, 1H), 1.27 (m, 1H), 0.77 (dd, 6H).

**Example Section E****Scheme 1**

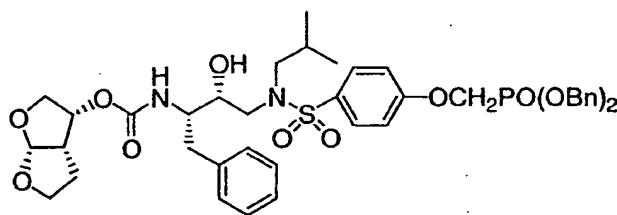


Example 1

To a solution of phenol 3 (336 mg, 0.68 mmol) in THF (10 mL) was added  $\text{Cs}_2\text{CO}_3$  (717 mg, 2.2 mmol) and triflate (636 mg, 1.5 mmol) in THF (3 mL). After the reaction mixture was stirred for 30 min at room temperature, the mixture was partitioned between EtOAc and water. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 40-50% EtOAc/hexane) to give dibenzylphosphonate 4 (420 mg, 80%) as a colorless oil.

Example 2

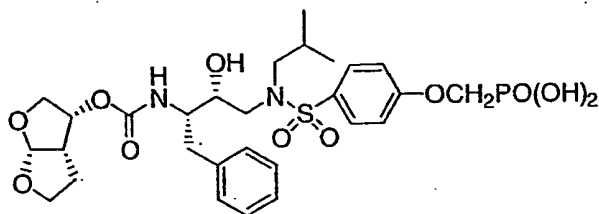
To a solution of dibenzylphosphonate 4 (420 mg, 0.548 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added TFA (0.21 mL, 2.74 mmol). After the reaction mixture was stirred for 2 h at room temperature, additional TFA (0.84 mL, 11 mmol) was added and the mixture was stirred for 3 h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between EtOAc and 1M  $\text{NaHCO}_3$ . The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to give amine 5 (325 mg, 89%).

Example 3

To a solution of carbonate (79 mg, 0.27 mmol), amine 5 (178 mg, 0.27 mmol), and  $\text{CH}_3\text{CN}$  (10 mL) was added DMAP (66 mg, 0.54 mmol) at  $0^\circ\text{C}$ . After the reaction mixture was warmed to room temperature and stirred for 16 hours, the mixture was concentrated under reduced pressure. The residue was chromatographed on silica gel (eluting 60-90% EtOAc/hexane) to give a mixture of carbamate 6 and starting carbonate. The mixture was further purified by HPLC on C18 reverse phase chromatography (eluting 60%  $\text{CH}_3\text{CN}$ /water)

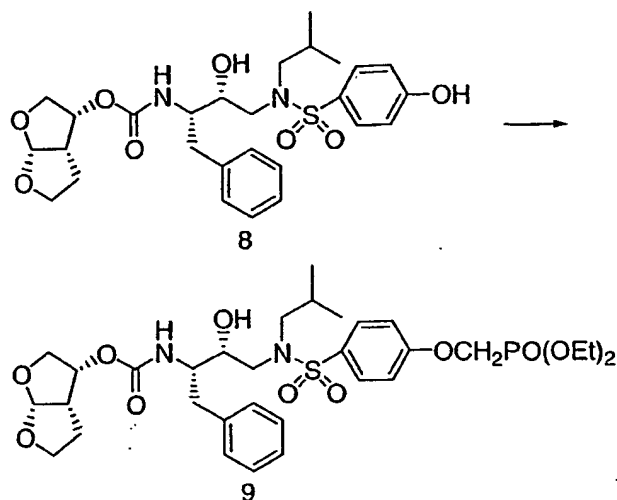
to give carbamate **6** (49 mg, 22%) as a colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.68 (d, 2H), 7.22 (m, 15 H), 6.95 (d, 2H), 5.62 (d, 1H), 5.15 (dt, 4H), 5.00 (m, 2H), 4.21 (d, 2H), 3.88 (m, 4H), 3.67 (m, 3H), 3.15 (m, 2H), 2.98 (m, 3H), 2.80 (m, 2H), 1.82 (m, 1H), 1.61 (m, 1H), 0.93 (d, 3H), 0.88 (d, 3H).

#### Example 4

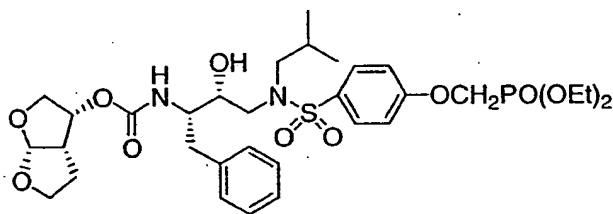


To a solution of carbamate **6** (21 mg, 0.026 mmol) in EtOH / EtOAc (2 mL/1 mL) was added 10% Pd/C (11 mg). After the reaction mixture was stirred under  $\text{H}_2$  atmosphere (balloon) for 2 hours, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give phosphonic acid **7** (17 mg, 100%) as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.73 (d, 2H), 7.19 (m, 5H), 7.13 (d, 2H), 5.53 (d, 1H), 4.26 (d, 2H), 3.86 (m, 1H), 3.64 (m, 5H), 3.38 (d, 1H), 3.13 (d, 1H), 3.03 (dd, 1H), 2.86 (m, 3H), 2.48 (m, 1H), 1.97 (m, 1H), 1.47 (m, 1H), 1.28 (m, 2H), 1.13 (t, 1H), 0.88 (d, 3H), 0.83 (d, 3H).

## Scheme 2

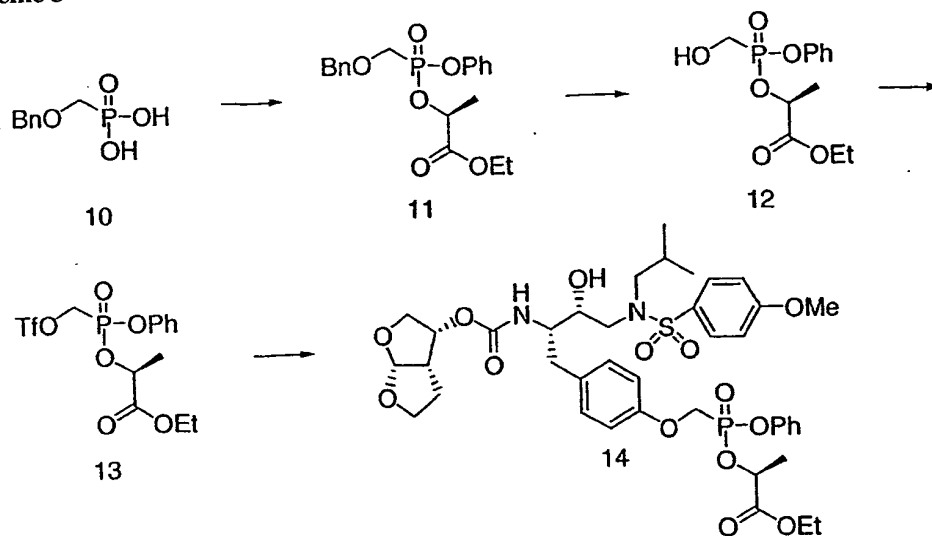
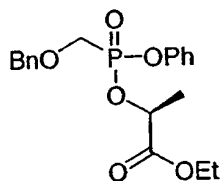


## Example 5

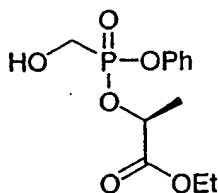


To a solution of phenol **8** (20 mg, 0.036 mmol) and triflate (22 mg, 0.073 mmol) in THF (2 mL) was added  $\text{Cs}_2\text{CO}_3$  (29 mg, 0.090 mmol). After the reaction mixture was stirred for 30 min at room temperature, the mixture was partitioned between EtOAc and water. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by preparative thin layer chromatography (eluting 80% EtOAc/hexane) to give diethylphosphonate **9** (21 mg, 83%) as a colorless oil.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.73 (d, 2H), 7.25 (m, 5H), 7.07 (d, 2H), 5.64 (d, 1H), 5.01 (m, 2H), 4.25 (m, 6H), 3.88 (m, 4H), 3.70 (m, 3H), 2.97 (m, 6H), 1.70 (m, 4H), 1.38 (t, 6H), 0.92 (d, 3H), 0.88 (d, 3H).  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  18.1.

Scheme 3

Example 6

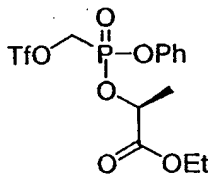
To a solution of phosphonic acid **10** (520 mg, 2.57 mmol) in CH<sub>3</sub>CN (5 mL) was added thionyl chloride (0.75 mL, 10.3 mmol) and heated to 70°C in an oil bath. After the reaction mixture was stirred for 2 h at 70°C, the mixture was concentrated and azeotroped with toluene. To a solution of the crude chloridate in toluene (5 mL) was added tetrazole (18 mg, 0.26 mmol) at 0°C. To this mixture was added phenol (121 mg, 1.28 mmol) and triethylamine (0.18 mL, 1.28 mmol) in toluene (3 mL) at 0°C. After the reaction mixture was warmed to room temperature and stirred for 2 h, ethyl lactate (0.29 mL, 2.57 mmol) and triethylamine (0.36 mL, 2.57 mmol) in toluene (2.5 mL) were added. The reaction mixture was stirred for 16 hours at room temperature, at which time the mixture was partitioned between EtOAc and sat. NH<sub>4</sub>Cl. The organic phase was washed with sat. NH<sub>4</sub>Cl, 1M NaHCO<sub>3</sub>, and brine, then dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 20-40% EtOAc/hexane) to give two diastereomers of phosphonate **11** (66 mg, 109 mg, 18% total) as colorless oils.

Example 7A

To a solution of phosphonate **11** isomer A (66 mg, 0.174 mmol) in EtOH (2 mL) was added 10% Pd/C (13 mg). After the reaction mixture was stirred under H<sub>2</sub> atmosphere (balloon) for 6 h, the mixture was filtered through Celite. The filtrate was evaporated under reduced pressure to give alcohol **12** isomer A (49 mg, 98%) as a colorless oil.

Example 7B

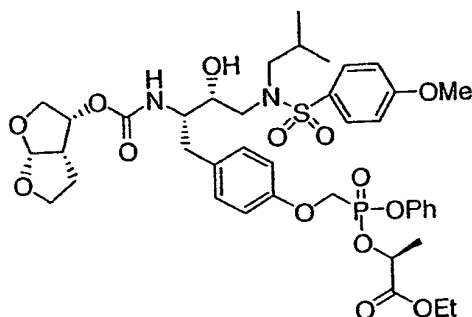
To a solution of phosphonate **11** isomer B (110 mg, 0.291 mmol) in EtOH (3 mL) was added 10% Pd/C (22 mg). After the reaction mixture was stirred under H<sub>2</sub> atmosphere (balloon) for 6 h, it was filtered through Celite. The filtrate was evaporated under reduced pressure to give alcohol **12** isomer B (80 mg, 95%) as a colorless oil.

Example 8A

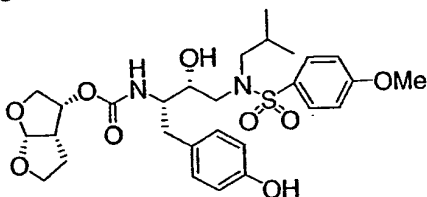
To a solution of alcohol **12** isomer A (48 mg, 0.167 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added 2,6-lutidine (0.03 mL, 0.250 mmol) and trifluoromethanesulfonic anhydride (0.04 mL, 0.217 mmol) at -40°C (dry ice-CH<sub>3</sub>CN bath). After the reaction mixture was stirred for 15 min at -40°C, the mixture was warmed to 0°C and partitioned between Et<sub>2</sub>O and 1M H<sub>3</sub>PO<sub>4</sub>. The organic phase was washed with 1M H<sub>3</sub>PO<sub>4</sub> (3 times), dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure to give triflate **13** isomer A (70 mg, 100%) as a pale yellow oil.

**Example 8B**

To a solution of alcohol **12** isomer B (80 mg, 0.278 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) was added 2,6-lutidine (0.05 mL, 0.417 mmol) and trifluoromethanesulfonic anhydride (0.06 mL, 0.361 mmol) at  $-40^\circ\text{C}$  (dry ice- $\text{CH}_3\text{CN}$  bath). After the reaction mixture was stirred for 15 min at  $-40^\circ\text{C}$ , the mixture was warmed to  $0^\circ\text{C}$  and partitioned between  $\text{Et}_2\text{O}$  and 1M  $\text{H}_3\text{PO}_4$ . The organic phase was washed with 1M  $\text{H}_3\text{PO}_4$  (3 times), dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to give triflate **13** isomer B (115 mg, 98%) as a pale yellow oil.

**Example 9A**

To a solution of phenol (64 mg, 0.111 mmol):

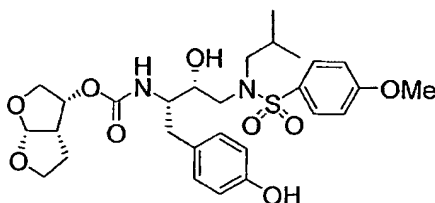


and triflate **13** isomer A (70 mg, 0.167 mmol) in THF (2 mL) was added  $\text{Cs}_2\text{CO}_3$  (72 mg, 0.222 mmol). After the reaction mixture was stirred for 30 min at room temperature, the mixture was partitioned between  $\text{EtOAc}$  and water. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 60-80%  $\text{EtOAc}$ /hexane) to give a mixture. The mixture was further purified by HPLC on C18 reverse phase chromatography (eluting 55%  $\text{CH}_3\text{CN}$ /water) to give phosphonate **14** isomer A (30 mg, 32%) as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.26 (m, 6H), 7.00 (m, 5H), 5.65 (d, 1H), 5.14 (m, 1H), 5.00 (m, 2H), 4.54 (dd, 1H), 4.44 (dd, 1H), 4.17 (m, 2H), 3.96 (dd, 1H), 3.86 (m, 5H), 3.72

(m, 3H), 3.14 (m, 1H), 2.97 (m, 4H), 2.79 (m, 2H), 1.83 (m, 1H), 1.62 (m, 3H), 1.50 (d, 3H), 1.25 (m, 3H), 0.93 (d, 3H), 0.88 (d, 3H).  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  17.4.

#### Example 9B

To a solution of phenol (106 mg, 0.183 mmol):



and triflate **13** isomer B (115 mg, 0.274 mmol) in THF (2 mL) was added  $\text{Cs}_2\text{CO}_3$  (119 mg, 0.366 mmol). After the reaction mixture was stirred for 30 min at room temperature, the mixture was partitioned between EtOAc and water. The organic phase was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (eluting 60-80% EtOAc/hexane) to give a mixture. The mixture was further purified by HPLC on C18 reverse phase chromatography (eluting 55%  $\text{CH}_3\text{CN}/\text{water}$ ) to give phosphonate **14** isomer B (28 mg, 18%) as a colorless solid.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.26 (m, 6H), 6.94 (m, 5H), 5.66 (d, 1H), 5.17 (m, 1H), 4.99 (m, 2H), 4.55 (m, 1H), 4.42 (m, 1H), 4.16 (m, 2H), 3.97 (m, 1H), 3.85 (m, 5H), 3.72 (m, 3H), 3.13 (m, 1H), 2.97 (m, 4H), 2.80 (m, 2H), 1.83 (m, 1H), 1.60 (m, 6H), 1.22 (m, 3H), 0.93 (d, 3H), 0.88 (d, 3H).  $^{31}\text{P}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  15.3.

#### Resolution of Compound 14 Diastereomers

Analysis was performed on an analytical Alltech Econosil column, conditions described below, with a total of about 0.5 mg **14** injected onto the column. This lot was a mixture of major and minor diastereomers where the lactate ester carbon is a mix of R and S configurations (fig. 1). Up to 2 mg could be resolved on the analytical column. Larger scale injections (up to 50 mg **14**) were performed on an Alltech Econosil semi-preparative column (fig. 2), conditions described below.

The isolated diastereomer fractions were stripped to dryness on a rotary evaporator under house vacuum, followed by a final high vacuum strip on a vacuum pump. The chromato-

graphic solvents were displaced by two portions of dichloromethane before the final high vacuum strip to aid in removal of trace solvents, and to yield a friable foam.

The bulk of the diastereomer resolution was performed with *n*-heptane substituted for hexanes for safety considerations.

Sample Dissolution: While a fairly polar solvent mixture is described below, the sample may be dissolved in mobile phase with a minimal quantity of ethyl alcohol added to dissolve the sample.

**Analytical Column, 0.45 mg Injection, Hexanes – IPA (90:10) (fig. 1)**

**HPLC CONDITIONS**

|                 |                                                                  |
|-----------------|------------------------------------------------------------------|
| Column          | : Alltech Econosil, 5 $\mu$ m, 4.6 x 250 mm                      |
| Mobile Phase    | : Hexanes – Isopropyl Alcohol (90:10)                            |
| Flow Rate       | : 1.5 mL/min                                                     |
| Run Time        | : 50 min                                                         |
| Detection       | : UV at 242 nm                                                   |
| Temperature     | : Ambient                                                        |
| Injection Size  | : 100 $\mu$ L                                                    |
| Sample Prep.    | : ~ 5 mg/mL, dissolved in hexanes –<br>ethyl alcohol (75:25)     |
| Retention Times | : 14 ~ 22 min<br>: 14 ~ 29 min<br>: Less Polar Impurity ~ 19 min |

**Semi-Preparative Column, 50 mg Injection, *n*-Heptane – IPA (84:16) (fig. 2)**



**HPLC CONDITIONS**

|                 |                                                                        |
|-----------------|------------------------------------------------------------------------|
| Column          | : Alltech Econosil, 10 $\mu$ m, 22 x 250 mm                            |
| Mobile Phase    | : <i>n</i> -Heptane – Isopropyl Alcohol (84:16)                        |
| Flow Rate       | : 10 mL/min                                                            |
| Run Time        | : 65 min                                                               |
| Detection       | : UV at 257 nm                                                         |
| Temperature     | : Ambient                                                              |
| Injection Size  | : ~50 mg                                                               |
| Dissolution     | : 2 mL mobile phase plus ~ 0.75 mL ethyl alcohol                       |
| Retention Times | : 14 ~ 41 min<br>: 14 ~ 54 min<br>: Less Polar Impurity ~ Not resolved |

**Example Section F****Example 1**

Phosphonic acid 2: To a solution of compound 1 (A. Flohr et al, J. Med. Chem., 42, 12, 1999; 2633-2640) (4.45 g, 17 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at room temperature was added bromotrimethylsilane (1.16 mL, 98.6 mmol). The solution was stirred for 19 h. The volatiles were evaporated under reduced pressure to give the oily phosphonic acid 2 (3.44 g, 100%).  
<sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.30 (m, 5H), 4.61 (s, 2 H), 3.69 (d, 2H).

**Example 2**

Compound 3: To a solution of phosphonic acid 2 (0.67 g, 3.3 mmol) in CH<sub>3</sub>CN (5 mL) was added thionyl chloride (1 mL, 13.7 mmol) and the solution was heated at 70°C for 2.5 h. The volatiles were evaporated under reduced pressure and dried in vacuo to afford an oily phosphonyl dichloride. The crude chloride intermediate was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and cooled in an ice/water bath. Ethyl lactate (1.5 mL, 13.2 mmol) and triethyl amine (1.8 mL, 13.2 mmol) were added dropwise. The mixture was stirred for 4 h at room temperature and diluted with more CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The organic solution was washed with 0.1N HCl, saturated aqueous NaHCO<sub>3</sub>, and brine, dried (MgSO<sub>4</sub>) filtered and evaporated under reduced

pressure. The crude product was chromatographed on silica gel to afford oily compound 3 (0.548 g, 41%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.30 (m, 5H), 5.00-5.20 (m, 2H), 4.65 (m, 2H), 4.20 (m, 4H), 3.90 (d, 2H), 1.52 (t, 6H), 1.20 (t, 6H).

5 Example 3

Alcohol 4: A solution of compound 3 (0.54 g, 1.34 mmol) in EtOH (15 mL) was treated with 10% Pd/C (0.1 g) under  $\text{H}_2$  (100 psi) for 4 h. The mixture was filtered and the filtrate was treated with fresh 10% PD/C (0.1 g) under  $\text{H}_2$  (1 atmosphere) for 18 h. The mixture was filtered and the filtrate was evaporated to afford alcohol 4 (0.395 g, 94%) as an oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  4.90-5.17 (m, 2H), 4.65 (q, 2H), 4.22 (m, 4H), 4.01 (m, 2H), 1.55 (t, 6H), 1.21 (t, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.8.

Example 4

Triflate 5: To a solution of alcohol 4 (122.8 mg, 0.393 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at  $-40^\circ\text{C}$  were added 2,6-lutidine (0.069 mL, 0.59 mmol) and trifluoromethansulfonic anhydride (0.086 mL, 0.51 mmol). Stirring was continued at  $0^\circ\text{C}$  for 2 h. and the mixture partitioned in  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic layer was washed with 0.1N HCl, saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product 5 (150 mg, 87%) was used for the next step without further purification.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  5.0-5.20 (m, 2H), 4.93 (d, 2H), 4.22 (m, 4H), 1.59 (m, 6H), 1.29 (t, 6H).

Example 5

Phosphonate 6: A solution of phenol 8 (see Scheme Section A, Scheme 1 and 2) (32 mg, 0.055 mmol) and triflate 5 (50 mg, 0.11 mmol) in THF (1.5 mL) at room temperature was treated with  $\text{Cs}_2\text{CO}_3$  (45.6 mg, 0.14 mmol). The mixture was stirred for 2.5 h and partitioned in EtOAc and saturated  $\text{NaHCO}_3$ . The organic layer was washed with 0.1N HCl, saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (30-70% EtOAc/hexane) affording the phosphonate 6 (41 mg, 84%) as a solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.13 (d, 2H), 7.00 (d, 2H), 6.90 (d, 2H), 5.65 (d, 1H), 4.90-5.22 (m, 3H), 4.40 (m, 2H), 4.20 (m, 4H), 3.90 (s, 3H), 3.65-4.00 (m, 5H), 2.70-3.20 (m, 6H), 1.52-1.87 (m, 12H), 1.25 (m, 6H), 0.85-0.90 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.0.

Example 6

Compound 7: To a solution of phosphonic acid 2 (0.48 g, 2.37 mmol) in CH<sub>3</sub>CN (4 mL) was added thionyl chloride (0.65 mL, 9.48 mmol) and the solution was heated at 70°C for 2.5 h. The volatiles were evaporated under reduced pressure and dried in vacuo to afford an oily phosphonyl dichloride. The crude chloride intermediate was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and cooled in an ice/water bath. Ethyl glycolate (0.9 mL, 9.5 mmol) and triethyl amine (1.3 mL, 9.5 mmol) were added dropwise. The mixture was stirred for 2 h at room temperature and diluted with more CH<sub>2</sub>Cl<sub>2</sub> (100 mL). The organic solution was washed with 0.1N HCl, saturated aqueous NaHCO<sub>3</sub>, and saturated NaCl, dried (MgSO<sub>4</sub>) filtered and concentrated under reduced pressure. The crude product was chromatographed on silica gel to afford oily compound 7 (0.223 g, 27%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.30 (m, 5H), 4.65 (m, 6H), 4.25 (q, 4H), 3.96 (d, 2H), 1.27 (t, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 24.0.

Example 7

Alcohol 8: A solution of compound 7 (0.22 g, 0.65 mmol) in EtOH (8 mL) was treated with 10% Pd/C (0.04 g) under H<sub>2</sub> (1 atmosphere) for 4 h. The mixture was filtered and the filtrate was evaporated to afford alcohol 8 (0.156 g, 96%) as an oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.66 (m, 4H), 4.23 (q, 4H), 4.06 (d, 2H), 1.55 (t, 6H), 1.26 (t, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 26.8.

Example 8

Triflate 9: To a solution of alcohol 8 (156 mg, 0.62 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at -40°C were added 2,6-lutidine (0.11 mL, 0.93 mmol) and trifluoromethanesulfonic anhydride (0.136 mL, 0.8 mmol). Stirring was continued at 0°C for 2 h. and the mixture partitioned in CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic layer was washed with 0.1N HCl, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product 9 (210 mg, 88%) was used for the next step without further purification. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 4.90 (d, 2H), 4.76 (d, 4H), 4.27 (q, 4H), 1.30 (t, 6H).

Example 9

Phosphonate 10: A solution of phenol 8 (30 mg, 0.052 mmol) and triflate 9 (30 mg, 0.078 mmol) in THF (1.5 mL) at room temperature was treated with Cs<sub>2</sub>CO<sub>3</sub> (34 mg, 0.1 mmol). The mixture was stirred for 2.5 h and partitioned in EtOAc and saturated NaHCO<sub>3</sub>. The organic layer was washed with 0.1N HCl, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and

evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (30-70% EtOAc/hexane) affording the unreacted phenol (xx) (12 mg, 40%) and the phosphonate 10 (16.6 mg, 38%) as a solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.13 (d, 2H), 7.00 (d, 2H), 6.90 (d, 2H), 5.65 (d, 1H), 5.00 (m, 2H), 4.75 (m, 4H), 4.48 (d, 2H), 4.23 (q, 4H), 3.90 (s, 3H), 3.65-4.00 (m, 5H), 2.70-3.20 (m, 6H), 2.23 (b.s., 2H), 1.52-1.87 (m, 4H), 1.25 (t, 6H), 0.85-0.90 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.0.

#### Example 10

Compound 11: To a solution of phosphonic acid 2 (0.512 g, 2.533 mmol) in  $\text{CH}_3\text{CN}$  (5 mL) was added thionyl chloride (0.74 mL, 10 mmol) and the solution was heated at  $70^\circ\text{C}$  for 2.5 h. The volatiles were evaporated under reduced pressure and dried in vacuo to afford an oily phophonyl dichloride. The crude chloride intermediate was dissolved in toluene (8 mL) and cooled in an ice/water bath. A catalytic amount of tetrazol (16 mg, 0.21 mmol) was added followed by the addition of a solution of triethylamine (0.35 mL, 2.53 mmol) and phenol (238 mg, 2.53 mmol) in toluene (5 mL). The mixture was stirred at room temperature for 3 h. A solution of ethyl glycolate (0.36 mL, 3.8 mmol) and triethyl amine (0.53 mL, 3.8 mmol) in toluene (3 mL) was added dropwise. The mixture was stirred for 18 h at room temperature and partitioned in EtOAc and 0.1N HCl. The organic solution was washed with saturated aqueous  $\text{NaHCO}_3$ , and saturated NaCl, dried ( $\text{MgSO}_4$ ) filtered and concentrated under reduced pressure. The crude product was chromatographed on silica gel to afford diphenyl phosphonate as a byproduct (130 mg) and compound 11 (0.16 g, 18%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.15-7.40 (m, 10H), 4.58-4.83 (m, 4H), 4.22 (q, 2H), 4.04 (dd, 2H), 1.24 (t, 3H).

#### Example 11

Alcohol 12: A solution of compound 11 (0.16 g, 0.44 mmol) in EtOH (5 mL) was treated with 10% Pd/C (0.036 g) under  $\text{H}_2$  (1 atmosphere) for 22 h. The mixture was filtered and the filtrate was evaporated to afford alcohol 12 (0.112 g, 93%) as an oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.15-7.36 (m, 5H), 4.81 (dd, 1H), 4.55 (dd, 1H), 4.22 (q, 2H), 4.12 (m, 2H), 3.78 (b.s., 1H), 1.26 (t, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.9

#### Example 12

Triflate 13: To a solution of alcohol 12 (112 mg, 0.41 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL) at  $-40^\circ\text{C}$  were added 2,6-lutidine (0.072 mL, 0.62 mmol) and trifluoromethansulfonic anhydride (0.09

mL, 0.53 mmol). Stirring was continued at 0°C for 3 h. and the mixture partitioned in CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic layer was washed with 0.1N HCl, saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (30% EtOAc/hexane) affording triflate 13 (106 mg, 64%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.36 (m, 2H), 7.25 (m, 3H), 4.80-5.10 (m, 3H), 4.60 (dd, 1H), 4.27 (q, 2H), 1.28 (t, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 11.1

### Example 13

Phosphonate 14: A solution of phenol 8 (32 mg, 0.052 mmol) and triflate 13 (32 mg, 0.079 mmol) in CH<sub>3</sub>CN (1.5 mL) at room temperature was treated with Cs<sub>2</sub>CO<sub>3</sub> (34 mg, 0.1 mmol). The mixture was stirred for 1 h and partitioned in EtOAc and saturated NaHCO<sub>3</sub>. The organic layer was washed with saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (70% EtOAc/hexane) affording phosphonate 14 (18 mg, 40%). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (d, 2H), 6.75-7.35 (m, 11H), 5.65 (d, 1H), 5.00 (m, 2H), 4.50-4.88 (m, 3H), 4.20 (q, 2H), 3.84 (s, 3H), 3.65-4.00 (m, 5H), 2.70-3.20 (m, 6H), 1.52-1.87 (m, 6H), 1.25 (t, 3H), 0.85-0.90 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.9, 17.7.

### Example 14

Piperidine 16: A solution of compound 15 (3.1 g, 3.673 mmol) in MeOH (100 mL) was treated with 10% Pd/C (0.35 g) under H<sub>2</sub> (1 atmosphere) for 18 h. The mixture was filtered and the filtrate was evaporated to afford phenol 16 (2 g, 88%). <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.76 (d, 2H), 7.08 (d, 2H), 7.04 (d, 2H), 6.65 (d, 2H), 5.59 (d, 1H), 4.95 (m, 1H), 3.98 (s, 3H), 3.65-4.00 (m, 5H), 3.30-3.50 (m, 3H), 2.80-3.26 (m, 5H), 2.40-2.70 (m, 3H), 1.35-2.00 (m, 7H), 1.16 (m, 2H); MS (ESI) 620 (M+H).

### Example 15

Formamide 17: Piperidine 16 obtained above (193 mg, 0.3118 mmol) in DMF (4 mL) was treated with formic acid (0.035 mL, 0.936 mmol), triethylamine (0.173 mL, 1.25 mmol) and EDCI (179 mg, 0.936 mmol) at room temperature. The mixture was stirred for 18 h and partitioned in EtOAc and saturated NaHCO<sub>3</sub>. The organic layer was washed with saturated NaCl, dried (MgSO<sub>4</sub>), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc/hexane) affording formamide 17 (162

mg, 80%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.96 (s, 1H), 7.68 (d, 2H), 7.04 (d, 2H), 6.97 (d, 2H), 6.76 (d, 2H), 5.63 (d, 1H), 5.37 (bs, 1H), 5.04 (m, 1H), 4.36 (m, 1H), 3.93 (s, 3H), 3.52-3.95 (m, 7H), 2.70-3.20 (m, 8H), 1.48-2.00 (m, 7H), 1.02 (m, 2H).

5 Example 16

Dibenzyl phosphonate 18: A solution of phenol 17 (123 mg, 0.19 mmol) and dibenzyl trifluoromethansulfonyloxymethanphosphonate YY (120 mg, 0.28 mmol) in  $\text{CH}_3\text{CN}$  (1.5 mL) at room temperature was treated  $\text{Cs}_2\text{CO}_3$  (124 mg, 0.38 mmol). The mixture was stirred for 3 h and partitioned in  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic layer was washed with

- 1) 0.1N HCl, saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) affording phosphonate 18 (154 mg, 88%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.96 (s, 1H), 7.68 (d, 2H), 7.35 (m, 10H), 7.10 (d, 2H), 6.97 (d, 2H), 6.80 (d, 2H), 5.63 (d, 1H), 4.96-5.24 (m, 6H), 4.37 (m, 1H), 4.20 (d, 2H), 3.84 (s, 3H), 3.52-3.95 (m, 7H), 2.55-3.20 (m, 8H), 1.48-2.00 (m, 7H), 1.02 (m, 2H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

Example 17

Phosphonic acid 19: A solution of phosphonate 18 (24 mg, 0.026 mmol) in MeOH (3 mL) was treated with 10% Pd/C (5 mg) under  $\text{H}_2$  (1 atmosphere) for 4 h. The mixture was filtered and the filtrate was evaporated to afford phosphonic acid 19 as a solid (18 mg, 93%).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.00 (s, 1H), 7.67 (d, 2H), 7.18 (d, 2H), 7.09 (d, 2H), 6.90 (d, 2H), 5.60 (d, 1H), 4.30 (m, 1H), 4.16 (d, 2H), 3.88 (s, 3H), 3.60-4.00 (m, 7H), 3.04-3.58 (m, 5H), 2.44-2.92 (m, 5H), 1.28-2.15 (m, 5H), 1.08 (m, 2H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.3.

Example 18

Diethyl phosphonate 20: A solution of phenol 17 (66 mg, 0.1 mmol) and diethyl trifluoromethansulfonyloxymethanphosphonate XY (46 mg, 0.15 mmol) in  $\text{CH}_3\text{CN}$  (1.5 mL) at room temperature was treated  $\text{Cs}_2\text{CO}_3$  (66 mg, 0.2 mmol). The mixture was stirred for 3 h and partitioned in  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic layer was washed with 0.1N HCl, saturated NaCl, dried ( $\text{MgSO}_4$ ), filtered and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) affording the unreacted 17 (17 mg, 26%) and diethyl phosphonate 20 (24.5 mg, 41%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$

8.00 (s, 1H), 7.70 (d, 2H), 7.16 (d, 2H), 7.00(d, 2H), 6.88 (d, 2H), 5.66 (d, 1H), 4.98-5.10 (m, 2H), 4.39 (m, 1H), 4.24 (m, 5H), 3.89 (s, 3H), 3.602-3.98 (m, 7H), 2.55-3.16 (m, 8H), 1.50-2.00 (m, 7H), 1.36 (t, 6H), 1.08 (m, 2H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.2

5    Example 19

N-methyl piperidine diethyl phosphonate 21: A solution of compound 20 (22.2 mg, 0.0278 mmol) in THF (1.5 mL) at 0°C was treated with a solution of borane in THF (1M, 0.083 mL). The mixture was stirred for 2 h at room temperature and the starting material was consumed completely as monitored by TLC. The reaction mixture was cooled in an ice/water bath and  
1) excess methanol (1 mL) was added to quench the reaction. The solution was concentrated in vacuo and the crude product was chromatographed on silica gel with MeOH/EtOAc to afford compound 21 (7 mg, 32%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.70 (d, 2H), 7.16 (d, 2H), 7.00(d, 2H), 6.88 (d, 2H), 5.66 (d, 1H), 4.98-5.10 (m, 2H), 4.24 (m, 4H), 3.89 (s, 3H), 3.602-3.98 (m, 7H), 2.62-3.15 (m, 9H), 2.26 (s, 3H), 1.52-2.15 (m, 10H), 1.36 (t, 6H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.3  
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Example Section G

Example 1

1) Compound 1: To a solution of 4-nitrobenzyl bromide (21.6 g, 100 mmol) in toluene (100 mL) was added triethyl phosphite (17.15 mL, 100 mL). The mixture was heated at 120°C for 14 hrs. The evaporation under reduced pressure gave a brown oil, which was purified by flash column chromatography (hexane/EtOAc= 2/1 to 100 % EtOAc) to afford compound 1.

Example 2

Compound 2: To a solution of compound 1 (1.0 g) in ethanol (60 mL) was added 10% Pd-C (300 mg). The mixture was hydrogenated for 14 hrs. Celite was added and the mixture was stirred for 5 mins. The mixture was filtered through a pad of celite, and washed with ethanol. Concentration gave compound 2.

Example 3

Compound 3: To a solution of compound 3 (292 mg, 1.2 mmol) and aldehyde (111 mg, 0.2 mmol) in methanol (3 mL) was added acetic acid (48  $\mu\text{L}$ , 0.8 mmol). The mixture was stirred for 5 mins, and sodium cyanoborohydride (25 mg, 0.4 mmol) was added. The mixture

was stirred for 14 hrs, and methanol was removed under reduced pressure. Water was added, and was extracted with EtOAc. The organic phase was washed 0.5 N NaOH solution (1x), water (2x), and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3) gave compound 3.

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#### Example 4

Compound 4: To a solution of compound 3 (79 mg, 0.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred for 2 hrs, and solvents were evaporated under reduced pressure. Coevaporation with EtOAc and CH<sub>2</sub>Cl<sub>2</sub> gave an oil. The oil was dissolved in THF (1 mL) and tetrabutylammonium fluoride (0.9 mL, 0.9 mmol) was added. The mixture was stirred for 1 hr, and solvent was removed. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/7) gave compound 4.

#### Example 5

- Compound 5: To a solution of compound 4 (0.1 mmol) in acetonitrile (1 mL) at 0°C was added DMAP (22 mg, 0.18 mmol), followed by bisfurancarboxylate (27 mg, 0.09 mmol). The mixture was stirred for 3 hrs at 0°C, and diluted with EtOAc. The organic phase was washed with 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3 to 100/5) afford compound 5 (50 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.70 (2 H, d, J = 8.9 Hz), 7.11 (2 H, d, J = 8.5 Hz), 6.98 (2 H, d, J = 8.9 Hz), 6.61 (2 H, d, J = 8.5 Hz), 5.71 (1 H, d, J = 5.2 Hz), 5.45 (1 H, m), 5.13 (1 H, m), 4.0 (6 H, m), 3.98-3.70 (4 H, m), 3.86 (3 H, s), 3.38 (2 H, m), 3.22 (1 H, m), 3.02 (5 H, m), 2.8 (1 H, m), 2.0-1.8 (3 H, m), 1.26 (6 H, t, J = 7.0 Hz), 0.95 (3 H, d, J = 6.7 Hz), 0.89 (3 H, d, J = 6.7 Hz).

#### Example 6

Compound 6: To a solution of compound 5 (30 mg, 0.04 mmol) in MeOH (0.8 mL) was added 37% formaldehyde (30 µL, 0.4 mmol), followed by acetic acid (23 µL, 0.4 mmol). The mixture was stirred for 5 mins, and sodium cyanoborohydride (25 mg, 0.4 mmol) was added. The reaction mixture was stirred for 14 hrs, and diluted with EtOAc. The organic phase was washed 0.5 N NaOH solution (2x), water (2x), and brine, and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3) gave compound 6 (11 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.60 (2 H, d, J = 8.9 Hz), 7.17 (2 H, m), 6.95 (2 H, d, J = 8.9 Hz), 6.77 (2 H,



d, J = 8.5 Hz), 5.68 (1 H, d, J = 5.2 Hz), 5.21 (1 H, m), 5.09 (1 H, m), 4.01 (6 H, m), 3.87 (3 H, s), 3.8-3.3 (4 H, m), 3.1-2.6 (7 H, m), 2.90 (3 H, s), 1.8 (3 H, m), 1.25 (6 H, m), 0.91 (6 H, m).

5 Example 7

Compound 7: To a solution of compound 1 (24.6 g, 89.8 mmol) in acetonitrile (500 mL) was added TMSBr (36 mL, 269 mmol). The reaction mixture was stirred for 14 hrs, and evaporated under reduced pressure. The mixture was coevaporated with MeOH (2x), toluene (2x), EtOAc (2x), and CH<sub>2</sub>Cl<sub>2</sub> to give a yellow solid (20 g). To the suspension of above  
1 yellow solid (15.8 g, 72.5 mmol) in toluene (140 mL) was added DMF (1.9 mL), followed by thionyl chloride (53 mL, 725 mmol). The reaction mixture was heated at 60°C for 5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x), EtOAc, and CH<sub>2</sub>Cl<sub>2</sub> (2x) to afford a brown solid. To the solution of the brown solid in CH<sub>2</sub>Cl<sub>2</sub> at 0°C was added benzyl alcohol (29 mL, 290 mmol), followed by slow addition of  
pyridine (35 mL, 435 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO<sub>4</sub>. Concentration gave a dark oil, which was purified by flash column chromatography (hexanes/EtOAc = 2/1 to 1/1) to afford compound 7.

Example 8

Compound 8: To a solution of compound 7 (15.3 g) in acetic acid (190 mL) was added Zinc dust (20 g). The mixture was stirred for 14 hrs, and celite was added. The suspension was filtered through a pad of celite, and washed with EtOAc. The solution was concentrated under reduced pressure to dryness. The mixture was diluted with EtOAc, and was washed with 2N NaOH (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave compound 8 as an oil (15 g).

Example 9

Compound 9: To a solution of compound 8 (13.5 g, 36.8 mmol) and aldehyde (3.9 g, 7.0 mmol) in methanol (105 mL) was added acetic acid (1.68 mL, 28 mmol). The mixture was stirred for 5 mins, and sodium cyanoborohydride (882 mg, 14 mmol) was added. The mixture was stirred for 14 hrs, and methanol was removed under reduced pressure. Water

was added, and was extracted with EtOAc. The organic phase was washed 0.5 N NaOH solution (1x), water (2x), and brine (1x), and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/3$ ) gave compound 9 (6.0 g).

; Example 10

Compound 10: To a solution of compound 9 (6.2 g, 6.8 mmol) in  $\text{CH}_2\text{Cl}_2$  (100 mL) was added trifluoroacetic acid (20 mL). The mixture was stirred for 2 hrs, and solvents were evaporated under reduced pressure. Coevaporation with EtOAc and  $\text{CH}_2\text{Cl}_2$  gave an oil. The oil was dissolved in THF (1 mL) and tetrabutylammonium fluoride (0.9 mL, 0.9 mmol) was added. The mixture was stirred for 1 hr, and solvent was removed. Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/7$ ) gave compound 10.

Example 11

Compound 11: To a solution of compound 10 (5.6 mmol) in acetonitrile (60 mL) at  $0^\circ\text{C}$  was added DMAP (1.36g, 11.1 mmol), followed by bisfurancarboxylate (1.65 g, 5.6 mmol). The mixture was stirred for 3 hrs at  $0^\circ\text{C}$ , and diluted with EtOAc. The organic phase was washed with 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/3$  to  $100/5$ ) afford compound 11 (3.6 g):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.70 (2 H, d,  $J = 8.9$  Hz), 7.30 (10 H, m), 7.07 (2 H, m), 6.97 (2 H, d,  $J = 8.9$  Hz), 6.58 (2 H, d,  $J = 8.2$  Hz), 5.70 (1 H, d,  $J = 5.2$  Hz), 5.42 (1 H, m), 5.12 (1 H, m), 4.91 (4 H, m), 4.0-3.7 (6 H, m), 3.85 (3 H, s), 3.4 (2 H, m), 3.25 (1 H, m), 3.06 (2 H, d,  $J = 21$  Hz), 3.0 (3 H, m), 2.8 (1 H, m), 1.95 (1 H, m), 1.82 (2 H, m), 0.91 (6 H, m).

; Example 12

Compound 12: To a solution of compound 11 (3.6 g) in ethanol (175 mL) was added 10% Pd-C (1.5 g). The reaction mixture was hydrogenated for 14 hrs. The mixture was stirred with celite for 5 mins, and filtered through a pad of celite. Concentration under reduced pressure gave compound 12 as a white solid (2.8 g):  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  7.68 (2 H, m), 7.08 (2 H, m), 6.93 (2 H, m), 6.48 (2 H, m), 5.95 (1 H, m), 5.0 (2 H, m), 3.9-3.6 (6 H, m), 3.82 (3 H, s), 3.25 (3 H, m), 3.05 (4 H, m), 2.72 (2 H, d,  $J = 20.1$  Hz), 2.0-1.6 (3 H, m), 0.81 (6 H, m).

Example 13

Compound 13: Compound 12 (2.6 g, 3.9 mmol) and L-alanine ethyl ester hydrochloride (3.575 g, 23 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (20 mL) and diisopropylethylamine (4.1 mL, 23 mmol) was added. To above mixture was added a solution of Aldrithiol (3.46 g, 15.6 mmol) and triphenylphosphine (4.08 g, 15.6 g) in pyridine (20 mL). The reaction mixture was stirred for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with 0.5 N NaOH solution (2x), water (2x), and brine, and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave a yellow oil, which was purified by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/5 to 100/10) to afford compound 13 (750 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (2 H, d, J = 8.8 Hz), 7.13 (2 H, m), 6.98 (2 H, d, J = 8.8 Hz), 6.61 (2 H, d, J = 8.0 Hz), 5.71 (1 H, d, J = 5.2 Hz), 5.54 (1 H, m), 5.16 (1 H, m), 4.15 (6 H, m), 4.1-3.6 (6 H, m), 3.86 (3 H, s), 3.4-3.2 (3 H, m), 3.1-2.8 (8 H, m), 2.0 (1 H, m), 1.82 (2 H, m), 1.3 (12 H, m), 0.92 (6 H, m).

Example 14

Compound 14: To a solution of 4-hydroxypiperidine (19.5 g, 193 mmol) in THF at 0°C was added sodium hydroxide solution (160 mL, 8.10 g, 203 mmol), followed by di-tert-butyl dicarbonate (42.1 g, 193 mmol). The mixture was warmed to 25°C, and stirred for 12 hours. THF was removed under reduced pressure, and the aqueous phase was extracted with EtOAc (2x). The combined organic layer was washed with water (2x) and brine, and dried over MgSO<sub>4</sub>. Concentration gave a compound 14 as a white solid (35 g).

Example 15

Compound 15: To a solution of alcohol 14 (5.25 g, 25 mmol) in THF (100 mL) was added sodium hydride (1.2 g, 30 mmol, 60%). The suspension was stirred for 30 mins, and chloromethyl methyl sulfide (2.3 mL, 27.5 mmol) was added. Starting material alcohol 14 still existed after 12 hrs. Dimethyl sulfoxide (50 mL) and additional chloromethyl methyl sulfide (2.3 mL, 27.5 mmol) were added. The mixture was stirred for additional 3 hrs, and THF was removed under reduced pressure. The reaction was quenched with water, and extracted with ethyl acetate. The organic phase was washed with water and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 8/1) gave compound 15 (1.24 g).

Example 16

Compound 16: To a solution of compound 15 (693 mg, 2.7 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL) at  $-78^\circ\text{C}$  was added a solution of sulfonyl chloride (214  $\mu\text{L}$ , 2.7 mmol) in  $\text{CH}_2\text{Cl}_2$  (5 mL). The reaction mixture was kept at  $-78^\circ\text{C}$  for 3 hrs, and solvents were removed to give a white solid. The white solid was dissolved in toluene (7 mL), and triethyl phosphite (4.5 mL, 26.6 mmol) was added. The reaction mixture was heated at  $120^\circ\text{C}$  for 12 hrs. Solvent and excess reagent was removed under reduced pressure to give compound 16.

Example 17

Compound 17: To a solution of compound 17 (600 mg) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 2 hrs, and was concentrated under reduced pressure to give an oil. The oil was diluted with methylene chloride and base resin was added. The suspension was filtered and the organic phase was concentrated to give compound 17.

Example 18

Compound 18: To a solution of compound 17 (350 mg, 1.4 mmol) and aldehyde (100 mg, 0.2 mmol) in methanol (4 mL) was added acetic acid (156  $\mu\text{L}$ , 2.6 mmol). The mixture was stirred for 5 mins, and sodium cyanoborohydride (164 mg, 2.6 mmol) was added. The mixture was stirred for 14 hrs, and methanol was removed under reduced pressure. Water was added, and was extracted with EtOAc. The organic phase was washed 0.5 N NaOH solution (1x), water (2x), and brine (1x), and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/3$ ) gave compound 18 (62 mg).

Example 19

Compound 19: To a solution of compound 18 (62 mg, 0.08 mmol) in THF (3 mL) were added acetic acid (9  $\mu\text{L}$ , 0.15 mmol) and tetrabutylammonium fluoride (0.45 mL, 1.0 N, 0.45 mmol). The mixture was stirred for 3 hr, and solvent was removed. Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/5$ ) gave an oil. To a solution of above oil in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 1 hrs, and was concentrated under reduced pressure. Coevaporation with EtOAc and  $\text{CH}_2\text{Cl}_2$  gave compound 19.

Example 20

Compound 20: To a solution of compound 19 (55 mg 0.08 mmol) in acetonitrile (1 mL) at 0°C was added DMAP (20 mg, 0.16 mmol), followed by bisfurancarboxylate (24 mg, 0.08 mmol). The mixture was stirred for 3 hrs at 0°C, and diluted with EtOAc. The organic phase  
; was washed with 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3 to 100/5) afford compound 20 (46 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.70 (2 H, d, J = 8.9 Hz), 7.01 (2 H, d, J = 8.9 Hz), 5.73 (1 H, d, J = 5.1 Hz), 5.51 (1 H, m), 5.14 (1 H, m), 4.16 (1 H, m), 4.06 (1 H, m), 3.94 (3 H, m), 3.86 (3 H, s), 3.80 (1 H, m), 3.75 (2 H, d, J = 9.1 Hz), 3.58 (1 H, m), 3.47 (1 H, m), 3.30 (1 H, m), 3.1-2.6 (8 H, m), 2.3 (2 H, m), 2.1-1.8 (5 H, m), 1.40 (2 H, m), 1.36 (6 H, t, J = 7.0 Hz), 0.93 (3 H, d, J = 6.7 Hz), 0.86 (3 h, d, J = 6.7 Hz).

Example 21

Compound 21: Compound 21 was made from Boc-4-Nitro-L-Phenylalanine (Fluka)  
; following the procedure for Compound 2 in Scheme Section A, Scheme 1.

Example 22

Compound 22: To a solution of chloroketone 21 (2.76 g, 8 mmol) in THF (50 mL) and water (6 mL) at 0°C (internal temperature) was added solid NaBH<sub>4</sub> (766 mg, 20 mmol) in several  
; portions over a period of 15 min while maintaining the internal temperature below 5°C. The mixture was stirred for 1.5 hrs at 0°C and solvent was removed under reduced pressure. The mixture was quenched with saturated KHSO<sub>3</sub> and extracted with EtOAc. The organic phase was washed with water and brine, and dried over MgSO<sub>4</sub>. Concentration gave a solid, which was recrystallized from EtOAc/hexane (1/1) to afford the chloroalcohol 22 (1.72 g).

Example 23

Compound 23: To a suspension of chloroalcohol 22 (1.8 g, 5.2 mmol) in EtOH (50 mL) was added a solution of KOH in ethanol (8.8 mL, 0.71 N, 6.2 mmol). The mixture was stirred for 2 h at room temperature and ethanol was removed under reduced pressure. The reaction  
; mixture was diluted with EtOAc, and washed with water (2x), saturated NH<sub>4</sub>Cl (2x), water, and brine, and dried over MgSO<sub>4</sub>. Concentration under reduced pressure afforded epoxide 23 (1.57g) as a white crystalline solid.

Example 24

Compound 24: To a solution of epoxide 23 (20 g, 65 mmol) in 2-propanol (250 mL) was added isobutylamine (65 mL) and the solution was refluxed for 90 min. The reaction mixture was concentrated under reduced pressure and was coevaporated with MeOH, CH<sub>3</sub>CN, and CH<sub>2</sub>Cl<sub>2</sub> to give a white solid. To a solution of the white solid in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) at 0°C was added triethylamine (19 mL, 136 mmol), followed by the addition of 4-methoxybenzenesulfonyl chloride (14.1 g, 65 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL). The reaction mixture was stirred at 0°C for 30 min, and warmed to room temperature and stirred for additional 2 hrs. The reaction solution was concentrated under reduced pressure and was diluted with EtOAc. The organic phase was washed with saturated NaHCO<sub>3</sub>, water and brine, and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave compound 24 as a white solid (37.5 g).

Example 25

Compound 25: To a solution of compound 24 (37.5 g, 68 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) at 0°C was added a solution of tribromoborane in CH<sub>2</sub>Cl<sub>2</sub> (340 mL, 1.0 N, 340 mmol). The reaction mixture was kept at 0°C for 1 hr, and warmed to room temperature and stirred for additional 3 hrs. The mixture was cooled to 0°C, and methanol (200 mL) was added slowly. The mixture was stirred for 1 hr and solvents were removed under reduced pressure to give a brown oil. The brown oil was coevaporated with EtOAc and toluene to afford compound 25 as a brown solid, which was dried under vacuum for 48 hrs.

Example 26

Compound 26: To a solution of compound 25 in THF (80 mL) was added a saturated sodium bicarbonate solution (25 mL), followed by a solution of Boc<sub>2</sub>O (982 mg, 4.5 mmol) in THF (20 mL). The reaction mixture was stirred for 5 hrs. THF was removed under reduced pressure, and aqueous phase was extracted with EtOAc. The organic phase was washed with water (2x) and Brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 1/1) gave compound 26 (467 mg).

Example 27

Compound 27: To a solution of compound 26 (300 mg, 0.56 mmol) in THF (6 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (546 mg, 1.68 mmol), followed by a solution of triflate (420 mg, 1.39 mmol)

in THF (2 mL). The reaction mixture was stirred for 1.5 hrs. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 1/1 to 1/3) gave compound 27 (300 mg).

i Example 28

Compound 28: To a solution of compound 27 (300 mg, 0.38 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 2.5 hrs, and was concentrated under reduced pressure. The mixture was diluted with EtOAc and was washed with 0.5 N NaOH solution (3x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Concentration gave a white solid. To the solution of above white solid in acetonitrile (3 mL) at 0°C was added DMAP (93 mg, 0.76 mmol), followed by bisfurancarboxylate (112 mg, 0.38 mmol). The mixture was stirred for 3 hrs at 0°C, and diluted with EtOAc. The organic phase was washed with 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>.

Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3 to 100/5) afford

- i compound 28 (230 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.16 (2 H, d, J = 8.5 Hz), 7.73 (2 H, d, J = 9.2 Hz), 7.42 (2 H, d, J = 8.5 Hz), 7.10 (2 H, d, J = 9.2 Hz), 5.65 (1 H, d, J = 4.8 Hz), 5.0 (2 H, m), 4.34 (2 H, d, J = 10 Hz), 4.25 (4 H, m), 4.0-3.6 (6 H, m), 3.2-2.8 (7 H, m), 1.82 (1 H, m), 1.6 (2 H, m), 1.39 (6 H, t, J = 7.0 Hz), 0.95 (6 H, m).

i Example 29

Compound 29: To a solution of compound 28 (50 mg) in ethanol (5 mL) was added 10% Pd-C (20 mg). The mixture was hydrogenated for 5 hrs. Celite was added, and the mixture was stirred for 5 mins. The reaction mixture was filtered through a pad of celite. Concentration under reduced pressure gave compound 29 (50 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (2 H, d, J = 8.8 Hz), 7.07 (2 H, 2 H, d, J = 8.8 Hz), 7.00 (2 H, d, J = 8.5 Hz), 6.61 (2 H, d, J = 8.5 Hz), 5.67 (1 H, d, J = 5.2 Hz), 5.05 (1 H, m), 4.90 (1 H, m), 4.34 (2 H, d, J = 10.3 Hz), 4.26 (2 H, m), 4.0-3.7 (6 H, m), 3.17 (1 H, m), 2.95 (4 H, m), 2.75 (2 H, m), 1.82 (1 H, m), 1.65 (2 H, m), 1.39 (6 H, t, J = 7.0 Hz), 0.93 (3 h, d, J = 6.4 Hz), 0.87 (3 h, d, J = 6.4 Hz).

i Example 30

Compound 30: To a solution of compound 29 (50 mg, 0.07 mmol) and formaldehyde (52 µL, 37%, 0.7 mmol) in methanol (1 mL) was added acetic acid (40 µL, 0.7 mmol). The mixture was stirred for 5 mins, and sodium cyanoborohydride (44 mg, 0.7 mmol) was added. The

mixture was stirred for 14 hrs, and methanol was removed under reduced pressure. Water was added, and was extracted with EtOAc. The organic phase was washed 0.5 N NaOH solution (1x), water (2x), and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/3) gave compound 30 (40 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.73 (2 H, d, J = 8.9 Hz), 7.10 (4 H, m), 6.66 (2 H, d, J = 8.2 Hz), 5.66 (1 H, d, J = 5.2 Hz), 5.02 (1 H, m), 4.88 (1 H, m), 4.32 (2 H, d, J = 10.1 Hz), 4.26 (4 H, m), 3.98 (1 H, m), 3.85 (3 H, m), 3.75 (2 H, m), 3.19 (1 H, m), 2.98 (4 H, m), 2.93 (6 H, s), 2.80 (2 H, m), 1.82 (1 H, m), 1.62 (2 H, m), 1.39 (6 H, t, J = 7.0 Hz), 0.90 (6 H, m).

#### Example 31

Compound 31: To a suspension of compound 25 (2.55 g, 5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) at 0°C was added triethylamine (2.8 mL, 20 mmol), followed by TMSCl (1.26 mL, 10 mmol). The mixture was stirred at 0°C for 30 mins, and warmed to 25°C and stirred for additional 1 hr. Concentration gave a yellow solid. The yellow solid was dissolved in acetonitrile (30 mL) and cooled to 0°C. To this solution was added DMAP (1.22 g, 10 mmol) and Bisfurancarboxylate (1.48 g, 5 mmol). The reaction mixture was stirred at 0°C for 2 hrs and for additional 1 hr at 25°C. Acetonitrile was removed under reduced pressure. The mixture was diluted with EtOAc, and washed with 5% citric acid (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Concentration gave a yellow solid. The yellow solid was dissolved in THF (40 mL), and acetic acid (1.3 mL, 20 mmol) and tetrabutylammonium fluoride (8 mL, 1.0 N, 8 mmol) were added. The mixture was stirred for 20 mins, and THF was removed under reduced pressure. Purification by flash column chromatography (hexanes/EtOAc = 1/1) gave compound 31 (1.5 g).

#### Example 32

Compound 32: To a solution of compound 31 (3.04 g, 5.1 mmol) in THF (75 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (3.31 g, 10.2 mmol), followed by a solution of triflate (3.24 g, 7.65 mmol) in THF (2 mL). The reaction mixture was stirred for 1.5 hrs, and THF was removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 1/1 to 1/3) gave compound 32 (2.4 g): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.17 (2 H, d, J = 8.5 Hz), 7.70 (2 H, J = 9.2 Hz), 7.43 (2 H, d, J = 8.5 Hz), 7.37 (10 H, m), 6.99 (2 H, d, J = 9.2 Hz), 5.66 (1 H, d, J =



5.2 Hz), 5.15 (4 H, m), 5.05 (2 H, m), 4.26 (2 H, d,  $J = 10.2$  Hz), 3.9-3.8 (4 H, m), 3.75 (2 H, m), 3.2-2.8 (7 H, m), 1.82 (1 H, m), 1.62 (2 H, m), 0.92 (6 H, m).

#### Example 33

- Compound 33: To a solution of compound 32 (45 mg) in acetic acid (3 mL) was added zinc (200 mg). The mixture was stirred for 5 hrs. Celite was added, and the mixture was filtered and washed with EtOAc. The solution was concentrated to dryness and diluted with EtOAc. The organic phase was washed with 0.5 N NaOH solution, water, and brine, and dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{isopropanol} = 100/5$ ) gave compound 33 (25 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.67 (2 H, d,  $J = 8.8$  Hz), 7.36 (10 H, m), 6.98 (4 H, m), 6.60 (2 H, d,  $J = 8.0$  Hz), 5.67 (1 H, d,  $J = 4.9$  Hz), 5.12 (4 H, m), 5.05 (1 H, m), 4.90 (1 H, m), 4.24 (2 H, d,  $J = 10.4$  Hz), 4.0-3.6 (6 H, m), 3.12 (1 H, m), 3.95 (4 H, m), 2.75 (2 H, m), 1.80 (1 H, m), 1.2 (2 H, m), 0.9 (6 H, m).

#### Example 34

- Compound 34: To a solution of compound 32 (2.4 g) in ethanol (140 mL) was added 10% Pd-C (1.0 g). The mixture was hydrogenated for 14 hrs. Celite was added, and the mixture was stirred for 5 mins. The slurry was filtered through a pad of celite, and washed with pyridine. Concentration under reduced pressure gave compound 34:  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  7.67 (2 H, d,  $J = 8.9$  Hz), 7.14 (2 H, d,  $J = 8.9$  Hz), 6.83 (2 H, d,  $J = 8.0$  Hz), 6.41 (2 H, d,  $J = 8.0$  Hz), 5.51 (1 H, d,  $J = 5.2$  Hz), 5.0-4.8 (2 H, m), 4.15 (2 H, d,  $J = 10.0$  Hz), 3.9-3.2 (8 H, m), 3.0 (2 H, m), 2.8 (4 H, m), 2.25 (1 H, m), 1.4 (2 H, m), 0.8 (6 H, m).

#### Example 35

Compound 35: Compound 34 (1.62 g, 2.47 mmol) and L-alanine butyl ester hydrochloride (2.69 g, 14.8 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (12 mL) and diisopropylethylamine (2.6 mL, 14.8 mmol) was added. To above mixture was added a solution of Aldrithiol (3.29 g, 14.8 mmol) and triphenylphosphine (3.88 g, 14.8 g) in pyridine (12 mL). The reaction mixture was stirred for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with 0.5 N NaOH solution (2x), water (2x), and brine, and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave a yellow oil, which was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/5$  to  $100/15$ ) to afford compound 35 (1.17 g):  $^1\text{H}$

NMR (CDCl<sub>3</sub>)  $\delta$  7.70 (2 H, d, J = 8.6 Hz), 7.05 (2 H, d, J = 8.6 Hz), 6.99 (2 H, d, J = 8.0 Hz), 6.61 (2 H, d, J = 8.0 Hz), 5.67 (1 H, d, J = 5.2 Hz), 5.05 (1 H, m), 4.96 (1 H, m), 4.28 (2 H, m), 4.10 (6 H, m), 4.0-3.6 (6 H, m), 3.12 (2 H, m), 2.92 (3 H, m), 2.72 (2 H, m), 1.82 (1 H, m), 1.75-1.65 (2 H, m), 1.60 (4 H, m), 1.43 (6 H, m), 1.35 (4 H, m), 0.91 (12 H, m).

#### Example 36

Compound 37: Compound 36 (100 mg, 0.15 mmol) and L-alanine butyl ester hydrochloride (109 mg, 0.60 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (1 mL) and diisopropylethylamine (105  $\mu$ L, 0.6 mmol) was added. To above mixture was added a solution of Aldrithiol (100 mg, 0.45 mmol) and triphenylphosphine (118 mg, 0.45 mmol) in pyridine (1 mL). The reaction mixture was stirred for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2x), and brine, and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave an oil, which was purified by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/5 to 100/15) to afford compound 37 (21 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.71 (2 H, d, J = 8.8 Hz), 7.15 (2 H, d, J = 8.2 Hz), 7.01 (2 H, d, J = 8.8 Hz), 6.87 (2 H, d, J = 8.2 Hz), 5.66 (1 H, d, J = 5.2 Hz), 5.03 (1 H, m), 4.95 (1 H, m), 4.2-4.0 (8 H, m), 3.98 (1 H, m), 3.89 (3 H, s), 3.88-3.65 (5 H, m), 3.15 (1 H, m), 2.98 (4 H, m), 2.82 (2 H, m), 1.83 (1 H, m), 1.63 (4 H, m), 1.42 (6 H, m), 1.35 (4 H, m), 0.95 (12 H, m).

#### Example 37

Compound 38: Compound 36 (100 mg, 0.15 mmol) and L-leucine ethyl ester hydrochloride (117 mg, 0.60 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (1 mL) and diisopropylethylamine (105  $\mu$ L, 0.6 mmol) was added. To above mixture was added a solution of Aldrithiol (100 mg, 0.45 mmol) and triphenylphosphine (118 mg, 0.45 mmol) in pyridine (1 mL). The reaction mixture was stirred for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2x), and brine, and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave an oil, which was purified by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 100/5 to 100/15) to afford compound 38 (12 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.72 (2 H, d, J = 8.5 Hz), 7.14 (2 H, d, J = 8.0 Hz), 7.00 (2 H, d, J = 8.5 Hz), 6.86 (2 H, d, J = 8.0 Hz), 5.66 (1 H, d, J = 5.2 Hz), 5.05 (1 H, m), 4.95 (1 H, m), 4.2-4.0 (8 H, m), 4.0-3.68 (6 H, m), 3.88 (3 H, s),

3.2-2.9 (5 H, m), 2.80 (2 H, m), 1.80 (1 H, m), 1.65 (4 H, m), 1.65-1.50 (4 H, m), 1.24 (6 H, m), 0.94 (18 H, m).

#### Example 38

Compound 39: Compound 36 (100 mg, 0.15 mmol) and L-leucine butyl ester hydrochloride (117 mg, 0.60 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (1 mL) and diisopropylethylamine (105  $\mu$ L, 0.6 mmol) was added. To above mixture was added a solution of Aldrithiol (100 mg, 0.45 mmol) and triphenylphosphine (118 mg, 0.45 mmol) in pyridine (1 mL). The reaction mixture was stirred for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2x), and brine, and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave an oil, which was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/5$  to  $100/15$ ) to afford compound 39 (32 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (2 H, d,  $J = 8.8$  Hz), 7.15 (2 H, d,  $J = 8.0$  Hz), 7.0 (2 H, d,  $J = 8.8$  Hz), 6.89 (2 H, d,  $J = 8.0$  Hz), 5.66 (1 H, d,  $J = 4.3$  Hz), 5.07 (1 H, m), 4.94 (1 H, m), 4.2-4.0 (8 H, m), 3.89 (3 H, s), 4.0-3.6 (6 H, m), 3.2-2.9 (5 H, m), 2.8 (2 H, m), 1.81 (1 H, m), 1.78-1.44 (10 H, m), 1.35 (4 H, m), 0.95 (24 H, m).

#### Example 39

Compound 41: Compound 40 (82 mg, 0.1 mmol) and L-alanine isopropyl ester hydrochloride (92 mg, 0.53 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (1 mL) and diisopropylethylamine (136  $\mu$ L, 0.78 mmol) was added. To above mixture was added a solution of Aldrithiol (72 mg, 0.33 mmol) and triphenylphosphine (87 mg, 0.33 mmol) in pyridine (1 mL). The reaction mixture was stirred at  $75^\circ\text{C}$  for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2x), and brine, and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave an oil, which was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/1$  to  $100/3$ ) to afford compound 41 (19 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (2 H, d,  $J = 8.9$  Hz), 7.2-7.35 (5 H, m), 7.15 (2 H, m), 7.01 (2 H, d,  $J = 8.9$  Hz), 6.87 (2 H, m), 5.65 (1 H, d,  $J = 5.4$  Hz), 5.05-4.93 (2 H, m), 4.3 (2 H, m), 4.19 (1 H, m), 3.98 (1 H, m), 3.88 (3 H, s), 3.80 (2 H, m), 3.70 (3 H, m), 3.18 (1 H, m), 2.95 (4 H, m), 2.78 (2 H, m), 1.82 (1 H, m), 1.62 (2 H, m), 1.35 (3 H, m), 1.25-1.17 (6 H, m), 0.93 (3 H, d,  $J = 6.4$  Hz), 0.88 (3 H, d,  $J = 6.4$  Hz).

Example 40

Compound 42: Compound 40 (100 mg, 0.13 mmol) and L-glycine butyl ester hydrochloride (88 mg, 0.53 mmol) were coevaporated with pyridine (2x). The mixture was dissolved in pyridine (1 mL) and diisopropylethylamine (136  $\mu$ L, 0.78 mmol) was added. To above mixture was added a solution of Aldrithiol (72 mg, 0.33 mmol) and triphenylphosphine (87 mg, 0.33 mmol) in pyridine (1 mL). The reaction mixture was stirred at 75°C for 20 hrs, and solvents were evaporated under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2x), and brine, and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave an oil, which was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 100/1$  to  $100/3$ ) to afford compound 42 (18 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (2 H, d,  $J = 9.2$  Hz), 7.35-7.24 (5 H, m), 7.14 (2 H, m), 7.00 (2 H, d,  $J = 8.8$  Hz), 6.87 (2 H, m), 5.65 (1 H, d,  $J = 5.2$  Hz), 5.04 (1 H, m), 4.92 (1 H, m), 4.36 (2 H, m), 4.08 (2 H, m), 3.95 (3 H, m), 3.88 (3 H, s), 3.80 (2 H, m), 3.76 (3 H, m), 3.54 (1 H, m), 3.15 (1 H, m), 2.97 (4 H, m), 2.80 (2 H, m), 1.82 (1 H, m), 1.62 (4 H, m), 1.35 (2 H, m), 0.9 (9 H, m).

Example Section HExample 1

Sulfonamide 1: To a suspension of epoxide (20 g, 54.13 mmol) in 2-propanol (250 mL) was added isobutylamine (54 mL, 541 mmol) and the solution was refluxed for 30 min. The solution was evaporated under reduced pressure and the crude solid was dissolved in  $\text{CH}_2\text{Cl}_2$  (250 mL) and cooled to 0°C. Triethylamine (15.1 mL, 108.26 mmol) was added followed by the addition of 4-nitrobenzenesulfonyl chloride (12 g, 54.13 mmol) and the solution was stirred for 40 min at 0°C, warmed to room temperature for 2 h, and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexane to give the sulfonamide (30.59 g, 90%) as an off-white solid.

Example 2

Phenol 2: A solution of sulfonamide 1 (15.58 g, 24.82 mmol) in EtOH (450 mL) and  $\text{CH}_2\text{Cl}_2$  (60 mL) was treated with 10% Pd/C (6 g). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 24 h. The reaction mixture was filtered through a plug of celite and concentrated under reduced pressure. The crude product was purified by column

chromatography on silica gel (6% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the phenol (11.34 g, 90%) as a white solid.

### Example 3

- i Dibenzyolphosphonate 3: To a solution of phenol 2 (18.25 g, 35.95 mmol) in CH<sub>3</sub>CN (200 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (23.43 g, 71.90 mmol) and triflate (19.83 g, 46.74 mmol). The reaction mixture was stirred at room temperature for 1 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (2/1-EtOAc/hexane) to give the dibenzyolphosphonate (16.87 g, 60%) as a white solid.

### Example 4

Amine 4: A solution of dibenzyolphosphonate (16.87 g, 21.56 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) at 0°C was treated with trifluoroacetic acid (30 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2x), water (2x), saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure to give the amine (12.94 g, 88%) as a white solid.

### Example 5

Carbonate 5: To a solution of (S)-(+)-3-hydroxytetrahydrofuran (5.00 g, 56.75 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (80 mL) was added triethylamine (11.86 mL, 85.12 mmol) and bis(4-nitrophenyl)carbonate (25.90 g, 85.12 mmol). The reaction mixture was stirred at room temperature for 24 h and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The CH<sub>2</sub>Cl<sub>2</sub> layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2/1-EtOAc/hexane) to give the carbonate (8.62 g, 60%) as a pale yellow oil which solidified upon refrigerating.

### Example 6

Carbamate 6: Two methods have been used.

Method 1: To a solution of 4 (6.8 g, 9.97 mmol) and 5 (2.65 g, 10.47 mmol) in CH<sub>3</sub>CN (70 mL) at 0 °C was added 4-(dimethylamino)pyridine (2.44 g, 19.95 mmol). The reaction mixture was stirred at 0°C for 3 h and concentrated. The residue was dissolved in EtOAc and washed with 0.5 N NaOH, saturated NaHCO<sub>3</sub>, H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the carbamate (3.97 g, 50%) as a pale yellow solid.

Method 2: To a solution of 4 (6.0 g, 8.80 mmol) and 5 (2.34 g, 9.24 mmol) in CH<sub>3</sub>CN (60 mL) at 0°C was added 4-(dimethylamino)pyridine (0.22 g, 1.76 mmol) and N, N-diisopropylethylamine (3.07 mL, 17.60 mmol). The reaction mixture was stirred at 0°C for 1 h and warmed to room temperature overnight. The solvent was evaporated under reduced pressure. The crude product was dissolved in EtOAc and washed with 0.5 N NaOH, saturated NaHCO<sub>3</sub>, H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the carbamate (3.85 g, 55%) as a pale yellow solid.

#### Example 7

Phosphonic Acid 7: To a solution of 6 (7.52 g, 9.45 mmol) in MeOH (350 mL) was added 10% Pd/C (3 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 48 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (5.24 g, 90%) as a white solid.

#### Example 8

Cbz Amide 8: To a solution of 7 (5.23 g, 8.50 mmol) in CH<sub>3</sub>CN (50 mL) was added N, O-bis(trimethylsilyl)acetamide (16.54 mL, 68 mmol) and then heated to 70°C for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was co-evaporated with toluene and dried under vacuum to afford the silylated intermediate which was used directly without any further purification. To a solution of the silylated intermediate in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at 0°C was added pyridine (1.72 mL, 21.25 mmol) and benzyl chloroformate (1.33 mL, 9.35 mmol). The reaction mixture was stirred at 0°C for 1 h and warmed to room temperature overnight. A solution of MeOH (50 mL) and 1% aqueous HCl (150 mL) was added at 0°C and stirred for 30 min. CH<sub>2</sub>Cl<sub>2</sub> was added and two layers were

separated. The organic layer was dried with  $\text{Na}_2\text{SO}_4$ , filtered, concentrated, co-evaporated with toluene, and dried under vacuum to give the Cbz amide (4.46 g, 70%) as an off-white solid.

5 Example 9

Diphenylphosphonate 9: A solution of 8 (4.454 g, 5.94 mmol) and phenol (5.591 g, 59.4 mmol) in pyridine (40 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (4.903 g, 23.76 mmol) was added. The reaction mixture was stirred at 70°C for 4 h and cooled to room temperature. EtOAc was added and the side product 1,3-dicyclohexyl urea was filtered off.

- ) The filtrate was concentrated and dissolved in  $\text{CH}_3\text{CN}$  (20 mL) at 0°C. The mixture was treated with DOWEX 50W x 8-400 ion-exchange resin and stirred for 30 min at 0°C. The resin was filtered off and the filtrate was concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the diphenylphosphonate (2.947 g, 55%) as a white solid.

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Example 10

Monophosphonic Acid 10: To a solution of 9 (2.945 g, 3.27 mmol) in  $\text{CH}_3\text{CN}$  (25 mL) at 0°C was added 1N NaOH (8.2 mL, 8.2 mmol). The reaction mixture was stirred at 0°C for 1 h. DOWEX 50W x 8-400 ion-exchange resin was added and the reaction mixture was stirred

- ) for 30 min at 0°C. The resin was filtered off and the filtrate was concentrated and co-evaporated with toluene. The crude product was triturated with EtOAc/hexane (1/2) to give the monophosphonic acid (2.427 g, 90%) as a white solid.

Example 11

- ;) Cbz Protected Monophosphoamidate 11: A solution of 10 (2.421 g, 2.93 mmol) and L-alanine isopropyl ester hydrochloride (1.969 g, 11.73 mmol) in pyridine (20 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (3.629 g, 17.58 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl,  $\text{H}_2\text{O}$ , saturated  $\text{NaHCO}_3$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monoamidate (1.569 g, 57%) as a white solid.

Example 12

Monophosphoamidate 12: To a solution of 11 (1.569 g, 1.67 mmol) in EtOAc (80 mL) was added 10% Pd/C (0.47 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and the crude product was purified by column chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub> to 1-8% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophosphoamidate 12a (1.12 g, 83%, **GS 108577**, 1:1 diastereomeric mixture A/B) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.45 (dd, 2H), 7.41-7.17 (m, 7H), 6.88 (dd, 2H), 6.67 (d, J = 8.4 Hz, 2H), 5.16 (broad s, 1H), 4.95 (m, 1H), 4.37-4.22 (m, 5H), 3.82-3.67 (m, 7H), 2.99-2.70 (m, 6H), 2.11-1.69 (m, 3H), 1.38 (m, 3H), 1.19 (m, 6H), 0.92 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.5, 19.6. 12b (29 mg, 2%, **GS108578**, diastereomer A) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.43 (d, J = 7.8 Hz, 2H), 7.35-7.17 (m, 7H), 6.89 (d, J = 8.4 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 5.16 (broad s, 1H), 4.96 (m, 1H), 4.38-4.32 (m, 4H), 4.20 (m, 1H), 3.82-3.69 (m, 7H), 2.99-2.61 (m, 6H), 2.10 (m, 1H), 1.98 (m, 1H), 1.80 (m, 1H), 1.38 (d, J = 7.2 Hz, 3H), 1.20 (d, J = 6.3 Hz, 6H), 0.92 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.5. 12c (22 mg, 1.6%, **GS 108579**, diastereomer B) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.45 (d, J = 8.1 Hz, 2H), 7.36-7.20 (m, 7H), 6.87 (d, J = 8.7 Hz, 2H), 6.67 (d, J = 8.4 Hz, 2H), 5.15 (broad s, 1H), 4.95 (m, 1H), 4.34-4.22 (m, 5H), 3.83-3.67 (m, 7H), 2.99-2.64 (m, 6H), 2.11-1.68 (m, 3H), 1.33 (d, J = 6.9 Hz, 3H), 1.20 (d, J = 6.0 Hz, 6H), 0.92 (d, J = 6.3 Hz, 3H), 0.86 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 19.6.

Example 13

Sulfonamide 13: To a suspension of epoxide (1.67 g, 4.52 mmol) in 2-propanol (25 mL) was added isobutylamine (4.5 mL, 45.2 mmol) and the solution was refluxed for 30 min. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and cooled to 0°C. Triethylamine (1.26 mL, 9.04 mmol) was added followed by the treatment of 3-nitrobenzenesulfonyl chloride (1.00 g, 4.52 mmol). The solution was stirred for 40 min at 0°C, warmed to room temperature for 2 h, and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (1/1-EtOAc/hexane) to give the sulfonamide (1.99 g, 70%) as a white solid.



Example 14

Phenol 14: Sulfonamide 13 (1.50 g, 2.39 mmol) was suspended in HOAc (40 mL) and concentrated HCl (20 mL) and heated to reflux for 3 h. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The crude product was partitioned between 10% MeOH/CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic layers were washed with NaHCO<sub>3</sub>, H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to give a yellow solid. The crude product was dissolved in CHCl<sub>3</sub> (20 mL) and treated with triethylamine (0.9 mL, 6.45 mmol) followed by the addition of Boc<sub>2</sub>O (0.61 g, 2.79 mmol). The reaction mixture was stirred at room temperature for 6 h. The product was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The CHCl<sub>3</sub> layer was washed with NaHCO<sub>3</sub>, H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1-5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the phenol (0.52 g, 45%) as a pale yellow solid.

Example 15

Dibenzylphosphonate 15: To a solution of phenol 14 (0.51 g, 0.95 mmol) in CH<sub>3</sub>CN (8 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (0.77 g, 2.37 mmol) and triflate (0.8 g, 1.90 mmol). The reaction mixture was stirred at room temperature for 1.5 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the dibenzylphosphonate (0.62 g, 80%) as a white solid.

Example 16

Amine 16: A solution of dibenzylphosphonate 15 (0.61 g, 0.75 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (8 mL) at 0°C was treated with trifluoroacetic acid (2 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2x), water (2x), saturated NaCl, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and evaporated under reduced pressure to give the amine (0.48 g, 90%) which was used directly without any further purification.

Example 17

Carbamate 17: To a solution of amine 16 (0.48 g, 0.67 mmol) in CH<sub>3</sub>CN (8 mL) at 0°C was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (0.2 g, 0.67 mmol, prepared according to Ghosh et al. J. Med. Chem. 1996, 39, 3278.) and 4-(dimethylamino)pyridine (0.17 g, 1.34 mmol). After stirring for 2 h at 0°C, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5N NaOH (2 x), 5% citric acid (2 x), saturated NaHCO<sub>3</sub>, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the carbamate (0.234 g, 40%) as a white solid.

Example 18

Aniline 18: To a solution of carbamate 17 (78 mg, 0.09 mmol) in 2 mL HOAc was added zinc powder. The reaction mixture was stirred at room temperature for 1.5 h and filtered through a small plug of celite. The filtrate was concentrated and co-evaporated with toluene. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the aniline (50 mg, 66%) as a white solid.

Example 19

Phosphonic Acid 19: To a solution of aniline (28 mg, 0.033mmol) in MeOH (1 mL) and HOAc (0.5 mL) was added 10% Pd/C (14 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 6 h. The reaction mixture was filtered through a small plug of celite. The filtrate was concentrated, co-evaporated with toluene, and dried under vacuum to give the phosphonic acid (15 mg, 68%, GS 17424) as a white solid: <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 7.16-6.82 (m, 8H), 5.50 (d, 1H), 4.84 (m, 1H), 3.86-3.37 (m, 9H), 2.95-2.40 (m, 6H), 1.98 (m, 1H), 1.42-1.23 (m, 2H), 0.84 (d, J = 6.3 Hz, 3H), 0.79 (d, J = 6.3 Hz, 3H). MS (ESI) 657 (M-H).

Example 20

Phenol 21: A suspension of aminohydrobromide salt 20 (22.75 g, 44 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (200 mL) at 0°C was treated with triethylamine (24.6 mL, 176 mmol) followed by slow addition of chlorotrimethylsilane (11.1 mL, 88 mmol). The reaction mixture was stirred at 0°C for 30 min and warmed to room temperature for 1 h. The solvent was removed under reduced

pressure to give a yellow solid. The crude product was dissolved in  $\text{CH}_2\text{Cl}_2$  (300 mL) and treated with triethylamine (18.4 mL, 132 mmol) and  $\text{Boc}_2\text{O}$  (12 g, 55 mmol). The reaction mixture was stirred at room temperature overnight. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The  $\text{CH}_2\text{Cl}_2$  layer was washed with  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was dissolved in THF (200 mL) and treated with 1.0 M TBAF (102 mL, 102 mmol) and HOAc (13 mL). The reaction mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1-3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the phenol (13.75 g, 58%) as a white solid.

#### Example 21

Dibenzylphosphonate 22: To a solution of phenol 21 (13.70 g, 25.48 mmol) in THF (200 mL) was added  $\text{Cs}_2\text{CO}_3$  (16.61 g, 56.96 mmol) and triflate (16.22 g, 38.22 mmol). The reaction mixture was stirred at room temperature for 1 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give the dibenzylphosphonate (17.59 g, 85%) as a white solid.

#### Example 22

Amine 23: A solution of dibenzylphosphonate 22 (17.58 g, 21.65 mmol) in  $\text{CH}_2\text{Cl}_2$  (60 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (30 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 1.5 h. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2x), water (2x), saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to give the amine (14.64 g, 95%) which was used directly without any further purification.

#### Example 23

Carbamate 24: To a solution of amine 23 (14.64 g, 20.57 mmol) in  $\text{CH}_3\text{CN}$  (200 mL) at  $0^\circ\text{C}$  was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (6.07 g, 20.57 mmol, prepared according to Ghosh et al., J. Med. Chem. 1996, 39, 3278.) and

4-(dimethylamino)pyridine (5.03 g, 41.14mmol). After stirring for 2 h at 0°C, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5N NaOH (2 x), 5% citric acid (2 x), saturated NaHCO<sub>3</sub>, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the carbamate (10 g, 56%) as a white solid.

#### Example 24

Phosphonic Acid 25: To a solution of carbamate 24 (8 g, 9.22 mmol) in EtOH (500 mL) was added 10% Pd/C (4 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 30 h. The reaction mixture was filtered through a plug of celite. The celite paste was suspended in pyridine and stirred for 30 min and filtered. This process was repeated twice. The combined solution was concentrated under reduced pressure to give the phosphonic acid (5.46 g, 90%) as an off-white solid.

#### Example 25

Cbz Amide 26: To a solution of 25 (5.26 g, 7.99 mmol) in CH<sub>3</sub>CN (50 mL) was added N, O-bis(trimethylsilyl)acetamide (15.6 mL, 63.92 mmol) and then heated to 70°C for 3 h. The reaction mixture was cooled to room temperature and concentrated. The residue was co-evaporated with toluene and dried under vacuum to afford the silylated intermediate which was used directly without any further purification. To a solution of the silylated intermediate in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at 0°C was added pyridine (1.49 mL, 18.38 mmol) and benzyl chloroformate (1.25mL, 8.79 mmol). The reaction mixture was stirred at 0°C for 1 h and warmed to room temperature overnight. A solution of MeOH (50 mL) and 1% aqueous HCl (150 mL) was added at 0°C and stirred for 30 min. CH<sub>2</sub>Cl<sub>2</sub> was added and two layers were separated. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, concentrated, co-evaporated with toluene, and dried under vacuum to give the Cbz amide (4.43 g, 70%) as an off-white solid.

#### Example 26

Diphenylphosphonate 27: A solution of 26 (4.43 g, 5.59 mmol) and phenol (4.21 g, 44.72 mmol) in pyridine (40 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (4.62 g, 22.36 mmol) was added. The reaction mixture was stirred at 70°C for 36 h and cooled to room temperature. EtOAc was added and the side product 1,3-dicyclohexyl urea was filtered

off. The filtrate was concentrated and dissolved in  $\text{CH}_3\text{CN}$  (20 mL) at  $0^\circ\text{C}$ . The mixture was treated with DOWEX 50W x 8-400 ion-exchange resin and stirred for 30 min at  $0^\circ\text{C}$ . The resin was filtered off and the filtrate was concentrated. The crude product was purified by column chromatography on silica gel (2/1-EtOAc/hexane to EtOAc) to give the diphenylphosphonate (2.11 g, 40%) as a pale yellow solid.

#### Example 27

Monophosphonic Acid 28: To a solution of 27 (2.11 g, 2.24 mmol) in  $\text{CH}_3\text{CN}$  (15 mL) at  $0^\circ\text{C}$  was added 1N NaOH (5.59 mL, 5.59 mmol). The reaction mixture was stirred at  $0^\circ\text{C}$  for 1 h. DOWEX 50W x 8-400 ion-exchange resin was added and the reaction mixture was stirred for 30 min at  $0^\circ\text{C}$ . The resin was filtered off and the filtrate was concentrated and co-evaporated with toluene. The crude product was triturated with EtOAc/hexane (1/2) to give the monophosphonic acid (1.75 g, 90%) as a white solid.

#### Example 28

Cbz Protected Monophosphoamidate 29: A solution of 28 (1.54 g, 1.77 mmol) and L-alanine isopropyl ester hydrochloride (2.38 g, 14.16 mmol) in pyridine (15 mL) was heated to  $70^\circ\text{C}$  and 1,3-dicyclohexylcarbodiimide (2.20 g, 10.62 mmol) was added. The reaction mixture was stirred at  $70^\circ\text{C}$  overnight and cooled to room temperature. The solvent was removed under reduced pressure and the residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl,  $\text{H}_2\text{O}$ , saturated  $\text{NaHCO}_3$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give the monophosphoamidate (0.70g, 40%) as an off-white solid.

#### Example 29

Monophosphoamidate 30a-b: To a solution of 29 (0.70 g, 0.71 mmol) in EtOH (10 mL) was added 10% Pd/C (0.3 g). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 6 h. The reaction mixture was filtered through a small plug of celite. The filtrate was concentrated and the crude products were purified by column chromatography on silica gel (7-10% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give the monoamidates 30a (0.106 g, 18%, GS 77369, 1/1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d,  $J$  = 8.7 Hz, 2H), 7.73-7.16 (m, 5H), 7.10-6.98 (m, 4H), 6.61 (d,  $J$  = 8.1 Hz, 2H), 5.67 (d,  $J$  = 4.8 Hz, 1H),

5.31-4.91 (m, 2H), 4.44 (m, 2H), 4.20 (m, 1H), 4.00-3.61 (m, 6H), 3.18-2.74 (m, 7H), 1.86-1.64 (m, 3H), 1.38 (m, 3H), 1.20 (m, 6H), 0.93 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.1, 18; MS(ESI) 869 (M+Na). 30b (0.200 g, 33%, GS 77425, 1/1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (dd, J = 8.7 Hz, J = 1.5 Hz, 2H), 7.36-7.16 (m, 5H), 7.09-7.00 (m, 4H), 6.53 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.4 Hz, 1H), 5.06-4.91 (m, 2H), 4.40 (m, 2H), 4.20 (m, 1H), 4.00-3.60 (m, 6H), 3.14 (m, 3H), 3.00-2.65 (m, 6H), 1.86-1.60 (m, 3H), 1.35 (m, 3H), 1.20 (m, 9H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.0, 17.9. MS (ESI) 897 (M+Na).

) Example 30

Synthesis of Bisamidates 32: A solution of phosphonic acid 31 (100 mg, 0.15 mmol) and L-valine ethyl ester hydrochloride (108 mg, 0.60 mmol) was dissolved in pyridine (5 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of  $\text{Ph}_3\text{P}$  (117 mg, 0.45 mmol) and 2,2'-dipyridyl disulfide (98 mg, 0.45 mmol) in pyridine (1 mL) followed by addition of N,N-diisopropylethylamine (0.1 mL, 0.60 mmol). The reaction mixture was stirred at room temperature for two days. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel to give the bisamidate (73 mg, 53%, GS 17389) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.86 (d, J = 8.1 Hz, 2H), 5.66 (d, J = 4.8 Hz, 1H), 5.05 (m, 1H), 4.95 (d, J = 8.7 Hz, 1H), 4.23-4.00 (m, 4H), 3.97-3.68 (m, 11H), 3.39-2.77 (m, 9H), 2.16 (m, 2H), 1.82-1.60 (m, 3H), 1.31-1.18 (m, 6H), 1.01-0.87 (m, 18H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  21.3; MS (ESI) 950 (M+Na).

Example 31

) Triflate 34: To a solution of phenol 33 (2.00 g, 3.46 mmol) in THF (15 mL) and  $\text{CH}_2\text{Cl}_2$  (5 mL) was added N-phenyltrifluoromethanesulfonimide (1.40 g, 3.92 mmol) and cesium carbonate (1.40 g, 3.92 mmol). The reaction mixture was stirred at room temperature overnight and concentrated. The crude product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give the triflate (2.09 g, 85%) as a white solid.

Example 32

Aldehyde 35: To a suspension of triflate 34 (1.45 g, 2.05 mmol), palladium (II) acetate (46 mg, 0.20 mmol) and 1,3-bis(diphenylphosphino)propane (84 mg, 0.2 mmol) in DMF (8 mL) under CO atmosphere (balloon) was slowly added triethylamine (1.65 mL, 11.87 mmol) and triethylsilane (1.90 mL, 11.87 mmol). The reaction mixture was heated to 70°C under CO atmosphere (balloon) and stirred overnight. The solvent was concentrated under reduced pressure and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the aldehyde (0.80 g, 66%) as a white solid.

Example 33

Substituted Benzyl Alcohol 36: To a solution of aldehyde 35 (0.80g, 1.35 mmol) in THF (9 mL) and H<sub>2</sub>O (1 mL) at -10°C was added NaBH<sub>4</sub> (0.13 g, 3.39 mmol). The reaction mixture was stirred for 1 h at -10°C and the solvent was evaporated under reduced pressure. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and washed with NaHSO<sub>4</sub>, H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (6% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the alcohol (0.56 g, 70%) as a white solid.

Example 34

Substituted Benzyl Bromide 37: To a solution of alcohol 36 (77 mg, 0.13 mmol) in THF (1 mL) and CH<sub>2</sub>Cl<sub>2</sub> (1 mL) at 0°C was added triethylamine (0.027 mL, 0.20 mmol) and methanesulfonyl chloride (0.011 mL, 0.14 mmol). The reaction mixture was stirred at 0°C for 30 min and warmed to room temperature for 3 h. Lithium bromide (60 mg, 0.69 mmol) was added and stirred for 45 min. The reaction mixture was concentrated and the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the bromide (60 mg, 70%).

Example 35

Diethylphosphonate 38: A solution of bromide 37 (49 mg, 0.075 mmol) and triethylphosphite (0.13 mL, 0.75 mmol) in toluene (1.5 mL) was heated to 120°C and stirred overnight. The reaction mixture was cooled to room temperature and concentrated under

reduced pressure. The crude product was purified by column chromatography on silica gel (6% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the diethylphosphonate (35 mg, 66%, GS 191338) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.27-7.16 (m, 4H), 7.00 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.00 (m, 2H), 4.04-3.73 (m, 13H), 3.13-2.80 (m, 9H), 1.82-1.64 (m, 3H), 1.25 (t, J = 6.9 Hz, 6H), 0.92 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) □ 26.4; MS (ESI) 735 (M+Na).

#### Example 36

N-tert-Butoxycarbonyl-O-benzyl-L-serine 39: To a solution of Boc-L-serine (15 g, 73.09 mmol) in DMF (300 mL) at 0°C was added NaH (6.43 g, 160.80 mmol, 60% in mineral oil) and stirred for 1.5 h at 0°C. After the addition of benzyl bromide (13.75 g, 80.40 mmol), the reaction mixture was warmed to room temperature and stirred overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in H<sub>2</sub>O. The crude product was partitioned between H<sub>2</sub>O and Et<sub>2</sub>O. The aqueous phase was acidified to pH<4 with 3 N HCl and extracted with EtOAc three times. The combined EtOAc solution was washed with H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated to give the N-tert-butoxycarbonyl-O-benzyl-L-serine (17.27 g, 80%).

#### Example 37

Diazo Ketone 40: To a solution of N-tert-Butoxycarbonyl-O-benzyl-L-serine 39 (10 g, 33.86 mmol) in dry THF (120 mL) at -15°C was added 4-methylmorpholine (3.8 mL, 34.54 mmol) followed by the slow addition of isobutylchloroformate (4.40 mL, 33.86 mmol). The reaction mixture was stirred for 30 min and diazomethane (~50 mmol, generated from 15 g Diazald according to Aldrichimica Acta 1983, 16, 3) in ether (~150 mL) was poured into the mixed anhydride solution. The reaction was stirred for 15 min and was then placed in an ice bath at 0°C and stirred for 1 h. The reaction was allowed to warm to room temperature and stirred overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in EtOAc, washed with water, saturated NaHCO<sub>3</sub>, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated. The crude product was purified by column chromatography (EtOAc/hexane) to afford the diazo ketone (7.50 g, 69%) as a yellow oil.



Example 38

Chloroketone 41: To a suspension of diazoketone 40 (7.50 g, 23.48 mmol) in ether (160 mL) at 0°C was added 4N HCl in dioxane (5.87 mL, 23.48 mmol). The reaction mixture was stirred at 0°C for 1 h. The reaction solvent was evaporated under reduced pressure to give the chloroketone which was used directly without any further purification.

Example 39

Chloroalcohol 42: To a solution of chloroketone 41 (7.70 g, 23.48 mmol) in THF (90 mL) was added water (10 mL) and the solution was cooled to 0°C. A solution of NaBH<sub>4</sub> (2.67 g, 70.45 mmol) in water (4 mL) was added dropwise over a period of 10 min. The mixture was stirred for 1 h at 0°C and saturated KHSO<sub>4</sub> was slowly added until the pH<4 followed by saturated NaCl. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (1/4 EtOAc/hexane) to give the chloroalcohol (6.20 g, 80%) as a diastereomeric mixture.

Example 40

Epoxide 43: A solution of chloroalcohol 42 (6.20 g, 18.79 mmol) in EtOH (150 mL) was treated with 0.71 M KOH (1.27 g, 22.55 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between EtOAc and water. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (1/6 EtOAc/hexane) to afford the desired epoxide 43 (2.79 g, 45%) and a mixture of diastereomers 44 (1.43 g, 23%).

Example 41

Sulfonamide 45: To a suspension of epoxide 43 (2.79 g, 8.46 mmol) in 2-propanol (30 mL) was added isobutylamine (8.40 mL, 84.60 mmol) and the solution was refluxed for 1 h. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and cooled to 0°C. Triethylamine (2.36 mL, 16.92 mmol) was added followed by the addition of 4-methoxybenzenesulfonyl chloride (1.75 g, 8.46 mmol). The solution was stirred for 40 min at 0°C, warmed to room temperature, and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic

phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was directly used without any further purification.

#### Example 42

- i Silyl Ether 46: A solution of sulfonamide 45 (5.10 g, 8.46 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was treated with triethylamine (4.7 mL, 33.82 mmol) and TMSOTf (3.88 mL, 16.91 mmol). The reaction mixture was stirred at room temperature for 1 h and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The aqueous phase was extracted twice with CH<sub>2</sub>Cl<sub>2</sub> and the combined organic extracts were washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (1/6 EtOAc/hexane) to give the silyl ether (4.50 g, 84%) as a thick oil.

#### Example 43

Alcohol 47: To a solution of silyl ether 46 (4.5 g, 7.14 mmol) in MeOH (50 mL) was added 10% Pd/C (0.5 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 2 h. The reaction mixture was filtered through a plug of celite and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the alcohol (3.40 g, 85%) as a white solid.

#### Example 44

Aldehyde 48: To a solution of alcohol 47 (0.60 g, 1.07 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) at 0°C was added Dess Martin reagent (0.77 g, 1.82 mmol). The reaction mixture was stirred at 0°C for 3 h and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and NaHCO<sub>3</sub>. The organic phase was washed with H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1/4 EtOAc/hexane) to give the aldehyde (0.45 g, 75%) as a pale yellow solid.

#### Example 45

Sulfonamide 50: To a suspension of epoxide (2.00 g, 5.41 mmol) in 2-propanol (20 mL) was added amine 49 (4.03 g, 16.23 mmol) (prepared in 3 steps starting from 4-(aminomethyl)piperidine according to Bioorg. Med. Chem. Lett., 2001, 11, 1261.). The

reaction mixture was heated to 80°C and stirred for 1 h. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and cooled to 0°C. Triethylamine (4.53 mL, 32.46 mmol) was added followed by the addition of 4-methoxybenzenesulfonyl chloride (3.36 g, 16.23 mmol). The solution was stirred for 40 min at 0°C, warmed to room temperature for 1.5 h, and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the sulfonamide (2.50 g, 59%).

#### Example 46

Amine 51: A solution of sulfonamide 50 (2.50 g, 3.17 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (6 mL) at 0°C was treated with trifluoroacetic acid (3 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 1.5 h. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2x), water (2x) and saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure to give the amine (1.96 g, 90%) which was used directly without any further purification.

#### Example 47

Carbamate 52: To a solution of amine 51 (1.96 g, 2.85 mmol) in CH<sub>3</sub>CN (15mL) at 0°C was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (0.84g, 2.85mmol, prepared according to Ghosh et al., J. Med. Chem. 1996, 39, 3278.) and 4-(dimethylamino)pyridine (0.70 g, 5.70 mmol). After stirring for 2 h at 0°C, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5N NaOH (2 x), 5% citric acid (2 x), saturated NaHCO<sub>3</sub>, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the carbamate (1.44 g, 60%) as a white solid.

**Example Section I****Example 1**

Carbonate 2: To a solution of (R)-(+)-3-hydroxytetrahydrofuran (1.23 g, 14 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added triethylamine (2.9 mL, 21 mmol) and bis(4-nitrophenyl)carbonate (4.7 g, 15.4 mmol). The reaction mixture was stirred at room temperature for 24 h and partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The CH<sub>2</sub>Cl<sub>2</sub> layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2/1-EtOAc/hexane) to give the carbonate (2.3 g, 65%) as a pale yellow oil which solidified upon standing.

**Example 2**

Carbamate 3: To a solution of 1 (0.385 g, 0.75 mmol) and 2 (0.210 g, 0.83 mmol) in CH<sub>3</sub>CN (7 mL) at room temperature was added N, N-diisopropylethylamine (0.16 mL, 0.90 mmol). The reaction mixture was stirred at room temperature for 44 h. The solvent was evaporated under reduced pressure. The crude product was dissolved in EtOAc and washed with saturated NaHCO<sub>3</sub>, brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1/1-EtOAc/hexane) to give the carbamate (0.322 g, 69%) as a white solid: mp 98-100°C (uncorrected).

**Example 3**

Phenol 4: To a solution of 3 (0.31 g, 0.49 mmol) in EtOH (10 mL) and EtOAc (5 mL) was added 10% Pd/C (30 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 15 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phenol (0.265 g) in quantitative yield.

**Example 4**

Diethylphosphonate 5: To a solution of phenol 4 (100 mg, 0.19 mmol) in THF (3 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (124 mg, 0.38 mmol) and triflate (85 mg, 0.29 mmol). The reaction mixture was stirred at room temperature for 4 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to

give the diethylphosphonate (63 mg, 49%, **GS 16573**) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.65 (d,  $J = 8.7\text{ Hz}$ , 2H), 7.21 (d,  $J = 8.7\text{ Hz}$ , 2H), 6.95 (d,  $J = 9\text{ Hz}$ , 2H), 6.84 (d,  $J = 8.4\text{ Hz}$ , 2H), 5.06 (broad s, 1H), 4.80 (d,  $J = 7.5\text{ Hz}$ , 1H), 4.19 (m, 6H), 3.83 (s, 3H), 3.80-3.70 (m, 6H), 3.09-2.72 (m, 6H), 2.00 (m, 1H), 1.79 (m, 2H), 1.32 (t,  $J = 7.5\text{ Hz}$ , 6H), 0.86 (d,  $J = 6.6\text{ Hz}$ , 3H), 0.83 (d,  $J = 6.6\text{ Hz}$ , 3H);  $^{31}\text{P}$  NMR  $\delta$  17.8.

#### Example 5

Dibenzylphosphonate 6: To a solution of phenol 4 (100 mg, 0.19 mmol) in THF (3 mL) was added  $\text{Cs}_2\text{CO}_3$  (137 mg, 0.42 mmol) and triflate (165 mg, 0.39 mmol). The reaction mixture was stirred at room temperature for 6 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the dibenzylphosphonate (130 mg, 84%, **GS 16574**) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.65 (d,  $J = 9\text{ Hz}$ , 2H), 7.30 (m, 10H), 7.08 (d,  $J = 8.4\text{ Hz}$ , 2H), 6.94 (d,  $J = 9\text{ Hz}$ , 2H), 6.77 (d,  $J = 8.7\text{ Hz}$ , 2H), 5.16-5.04 (m, 5H), 4.80 (d,  $J = 8.1\text{ Hz}$ , 1H), 4.16 (d,  $J = 10.2\text{ Hz}$ , 2H), 3.82 (s, 3H), 3.75-3.71 (m, 6H), 3.10-2.72 (m, 6H), 2.00 (m, 1H), 1.79 (m, 2H), 0.86 (d,  $J = 6.6\text{ Hz}$ , 3H), 0.83 (d,  $J = 6.6\text{ Hz}$ , 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  18.8.

#### Example 6

Phosphonic Acid 7: To a solution of 6 (66 mg, 0.08 mmol) in EtOH (3 mL) was added 10% Pd/C (12 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 15 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated under reduced pressure and triturated with EtOAc to give the phosphonic acid (40 mg, 78%, **GS 16575**) as a white solid.

#### Example 7

Carbonate 8: To a solution of (S)-(+)-3-hydroxytetrahydrofuran (2 g, 22.7 mmol) in  $\text{CH}_3\text{CN}$  (50 mL) was added triethylamine (6.75 mL, 48.4 mmol) and  $\text{N,N}'$ -disuccinimidyl carbonate (6.4 g, 25 mmol). The reaction mixture was stirred at room temperature for 5 h and concentrated under reduced pressure. The residue was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The organic phase was dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc as eluant)

followed by recrystallization (EtOAc/hexane) to give the carbonate (2.3 g, 44%) as a white solid.

#### Example 8

Carbamate 9: To a solution of 1 (0.218 g, 0.42 mmol) and 8 (0.12 g, 0.53 mmol) in CH<sub>3</sub>CN (3 mL) at room temperature was added N, N-diisopropylethylamine (0.11 mL, 0.63 mmol). The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase was washed with brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (1/1-EtOAc/hexane) to give the carbamate (0.176 g, 66%) as a white solid.

#### Example 9

Phenol 10: To a solution of 9 (0.176 g, 0.28 mmol) in EtOH (10 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phenol (0.151 g, GS 10) in quantitative yield.

#### Example 10

Diethylphosphonate 11: To a solution of phenol 10 (60 mg, 0.11 mmol) in THF (3 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (72 mg, 0.22 mmol) and triflate (66 mg, 0.22 mmol). The reaction mixture was stirred at room temperature for 4 h and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the diethylphosphonate (38 mg, 49%, GS 11) as a white solid.

#### Example Section J

##### Example 1

Triflate 1: To a solution of A (4 g, 6.9 mmol) in THF (30 mL) and CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (2.7 g, 8 mmol) and N-phenyltrifluoromethanesulfonimide (2.8 g, 8.0 mmol) and stirred at room temperature for 16 h. The reaction mixture was concentrated under

reduced pressure. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine twice. The organic phase was dried over sodium sulfate and used for next reaction without further purification.

### Example 2

Aldehyde 2: A solution of crude above triflate 1 (~6.9 mmol) in DMF (20 mL) was degassed (high vacuum for 5 min, argon purge, repeat 3 times). To this solution were quickly added  $\text{Pd}(\text{OAc})_2$  (120 mg, 266  $\mu\text{mol}$ ) and bis(diphenylphosphino-propane) (dppp, 220 mg, 266  $\mu\text{mol}$ ), and heated to 70°C. To this reaction mixture was rapidly introduced carbon monoxide, and stirred at room temperature under an atmospheric pressure of carbon monoxide, followed by slow addition of TEA (5.4 mL, 38 mmol) and triethylsilane (3 mL, 18 mmol). The resultant mixture was stirred at 70°C for 16 h, then cooled to room temperature, concentrated under reduced pressure, partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine. The organic phase was concentrated under reduced pressure and purified on silica gel column to afford aldehyde 2 (2.1 g, 51%) as white solid.

### Example 3

Compounds 3a-3e: Representative Procedure, 3c: A solution of aldehyde 2 (0.35 g, 0.59 mmol), L-alanine isopropyl ester hydrochloride (0.2 g, 1.18 mmol), glacial acetic acid (0.21 g, 3.5 mmol) in 1,2-dichloroethane (10 mL) was stirred at room temperature for 16 h, followed by addition of sodium cyanoborohydride (0.22 g, 3.5 mmol) and methanol (0.5 mL). The resulting solution was stirred at room temperature for one h. The reaction mixture was washed with sodium bicarbonate solution, saturated brine, and chromatographed on silica gel to afford 3c (0.17 g, 40%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.72 (d, 2H), 7.26 (d, 2H), 7.20 (d, 2H), 7.0 (d, 2H), 5.65 (d, 1H), 4.90-5.30 (m, 3H), 3.53-4.0 (m overlapping s, 13H), 3.31 (q, 1H), 2.70-3.20 (m, 7H), 1.50-1.85 (m, 3H), 1.25-1.31 (m, 9H), 0.92 (d, 3H), 0.88 (d, 3H). MS: 706 ( $M + 1$ ).

| Compound | R <sub>1</sub> | R <sub>2</sub> | Amino Acid |
|----------|----------------|----------------|------------|
| 3a       | Me             | Me             | Ala        |
| 3b       | Me             | Et             | Ala        |
| 3c       | Me             | iPr            | Ala        |
| 3d       | Me             | Bn             | Ala        |
| 3e       | iPr            | Et             | Val        |

Example 4

Sulfonamide 1: To a solution of crude amine A (1 g, 3 mmol) in  $\text{CH}_2\text{Cl}_2$  was added TEA (0.6 g, 5.9 mmol) and 3-methoxybenzenesulfonyl chloride (0.6 g, 3 mmol). The resulting solution was stirred at room temperature for 5 h, and evaporated under reduced pressure. The residue was chromatographed on silica gel to afford sulfonamide 1 (1.0 g, 67%).

Example 5

Amine 2: To a  $0^\circ\text{C}$  cold solution of sulfonamide 1 (0.85 g, 1.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (40 mL) was treated with  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  (10 mL of 1 M solution, 10 mmol). The solution was stirred at  $0^\circ\text{C}$  10 min and then warmed to room temperature and stirred for 1.5 h. The reaction mixture was quenched with  $\text{CH}_3\text{OH}$ , concentrated under reduced pressure, azeotroped with  $\text{CH}_3\text{CN}$  three times. The crude amine 2 was used for next reaction without further purification.

Example 6

Carbamate 3: A solution of crude amine 2 (0.83 mmol) in  $\text{CH}_3\text{CN}$  (20 mL) and was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (245 mg, 0.83 mmol, prepared according to Ghosh et al., J. Med. Chem. 1996, 39, 3278.) and N,N-dimethylaminopyridine (202 mg, 1.7 mmol). After stirring for 16 h at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$  three times. The organic phase was evaporated under reduced pressure. The residue was purified by chromatography on silica gel affording the carbamate 3 (150 mg, 33%) as a solid.

Example 7

Diethylphosphonate 4: To a solution of carbamate 3 (30 mg, 54  $\mu\text{mol}$ ) in THF (5 mL) was added  $\text{Cs}_2\text{CO}_3$  (54 mg, 164  $\mu\text{mol}$ ) and triflate # (33 mg, 109  $\mu\text{mol}$ ). After stirring the reaction mixture for 30 min at room temperature, additional  $\text{Cs}_2\text{CO}_3$  (20 mg, 61  $\mu\text{mol}$ ) and triflate (15 mg, 50  $\mu\text{mol}$ ) were added and the mixture was stirred for 1 more hour. The reaction mixture was evaporated under reduced pressure and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and water. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The crude product was chromatographed on silica gel and repurified by HPLC (50%  $\text{CH}_3\text{CN}$ -50%  $\text{H}_2\text{O}$  on C18 column) to give the diethylphosphonate 4 (15 mg,



39%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.45 (m, 3H), 7.17-7.30 (m, 6H), 5.64 (d, 1H), 5.10 (d, 1H), 5.02 (q, 1H), 4.36 (d, 2H), 4.18-4.29 (2 q overlap, 4H), 3.60-3.98 (m, 7H), 2.70-3.10 (m, 7H), 1.80-1.90 (m, 1H), 1.44-1.70 (m, 2H +  $\text{H}_2\text{O}$ ), 1.38 (t, 6H), 0.94 (d, 3H), 0.90 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 18.7 ppm; MS (ESI) 699 (M + H).

#### Example 8

Dibenzylphosphonate 5: To a solution of carbamate 3 (100 mg, 182  $\mu\text{mol}$ ) in THF (10 mL) was added  $\text{Cs}_2\text{CO}_3$  (180 mg, 550  $\mu\text{mol}$ ) and dibenzylhydroxymethyl phosphonate triflate, Section A, Scheme 2, Compound 9, (150 mg, 360  $\mu\text{mol}$ ). After stirring the reaction mixture for 1 h at room temperature, the reaction mixture was evaporated under reduced pressure and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and water. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The residue was purified by HPLC (50%  $\text{CH}_3\text{CN}$ -50%  $\text{H}_2\text{O}$  on C18 column) to give the dibenzylphosphonate 5 (110 mg, 72%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.41 (d, 2H), 7.35 (s, 10 H), 7.17-7.30 (m, 6H), 7.09-7.11 (m, 1H), 5.64 (d, 1H), 4.90-5.15 (m, 6H), 4.26 (d, 2H), 3.81-3.95 (m, 4H), 3.64-3.70 (m, 2H), 2.85-3.25 (m, 7H), 1.80-1.95 (m, 1H), 1.35-1.50 (m, 1H), 0.94 (d, 3H), 0.91 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.4 ppm; MS (ESI): 845 (M + Na), 1666 (2M + Na).

#### Example 9

Phosphonic acid 6: A solution of dibenzylphosphonate 5 (85 mg, 0.1 mmol) was dissolved in MeOH (10 mL) treated with 10% Pd/C (40 mg) and stirred under  $\text{H}_2$  atmosphere (balloon) overnight. The reaction was purged with  $\text{N}_2$ , and the catalyst was removed by filtration through celite. The filtrate was evaporated under reduced pressure to afford phosphonic acid 6 (67 mg, quantitatively).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.40-7.55 (m, 3H), 7.10-7.35 (m, 6H), 5.57 (d, 1H), 4.32 (d, 2H), 3.90-3.95 (m, 1H), 3.64-3.78 (m, 5H), 3.47 (m, 1H), 2.85-3.31 (m, 5H), 2.50-2.60 (m, 1H), 2.00-2.06 (m, 1H), 1.46-1.60 (m, 1H), 1.30-1.34 (m, 1H), 0.9 (d, 3H), 0.90 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ ): 16.60 ppm; MS (ESI): 641 (M - H).

#### Example 10

Sulfonamide 1: To a solution of crude amine A (0.67 g, 2 mmol) in  $\text{CH}_2\text{Cl}_2$  (50 mL) was added TEA (0.24 g, 24 mmol) and crude 3-acetoxy-4-methoxybenzenesulfonyl chloride (0.58 g, 2.1 mmol), was prepared according to Kratzl et al., Monatsh. Chem. 1952, 83, 1042-1043),

and the solution was stirred at room temperature for 4 h, and evaporated under reduced pressure. The residue was chromatographed on silica gel to afford sulfonamide 1 (0.64 g, 54%). MS: 587 (M + Na), 1150 (2M + Na)

- Phenol 2: Sulfonamide 1 (0.64 g, 1.1 mmol) was treated with saturated  $\text{NH}_3$  in MeOH (15 mL) at room temperature for 15 min., then evaporated under reduced pressure. The residue was purified on silica gel column to afford phenol 2 (0.57 g, 96%).

#### Example 11

- Dibenzylphosphonate 3a: To a solution of phenol 2 (0.3 g, 0.57 mmol) in THF (8 mL) was added  $\text{Cs}_2\text{CO}_3$  (0.55 g, 1.7 mmol) and dibenzylhydroxymethyl phosphonate triflate (0.5 g, 1.1 mmol). After stirring the reaction mixture for 1 h at room temperature, the reaction mixture was quenched with water and partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated ammonium chloride aqueous solution. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel (40% EtOAc/ 60% hexane) to give the dibenzylphosphonate 3a (0.36 g, 82%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.20-7.40 (m, 17H), 6.91 (d, 1H), 5.10-5.25 (2 q(ab) overlap, 4H), 4.58-4.70 (m, 1H), 4.34 (d, 2H), 3.66-3.87 (m + s, 5H), 2.85-3.25 (m, 6H), 1.80-1.95 (m, 1H), 1.58 (s, 9H), 0.86-0.92 (2d, 6H).

#### Example 12

Diethylphosphonate 3b: To a solution of phenol 2 (0.15 g, 0.28 mmol) in THF (4 mL) was added  $\text{Cs}_2\text{CO}_3$  (0.3 g, 0.92 mmol) and diethylhydroxymethyl phosphonate triflate (0.4 g, 1.3 mmol). After stirring the reaction mixture for 1 h at room temperature, the reaction mixture was quenched with water and partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$  aqueous solution. The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel (1%  $\text{CH}_3\text{OH}$ - $\text{CH}_2\text{Cl}_2$ ) to give the diethylphosphonate 3b (0.14 g, 73%).

#### Example 13

Amine 4a: To a solution of 3a (0.35 g, 0.44 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was treated with TFA (0.75 g, 6.6 mmol) at room temperature for 2 h. The reaction was evaporated under reduced

pressure, azeotroped with CH<sub>3</sub>CN twice, dried to afford crude amine 4a. This crude 4a was used for next reaction without further purification.

#### Example 14

- i) Amine 4b: To a solution of 3b (60 mg, 89  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was treated with TFA (0.1 mL, 1.2 mmol) at room temperature for 2 h. The reaction was evaporated under reduced pressure, azeotroped with CH<sub>3</sub>CN twice, dried to afford crude amine 4b (68 mg). This crude 4b was used for next reaction without further purification.

#### Example 15

Carbamate 5a: An ice-cold solution of crude amine 4a (0.44 mmol) in CH<sub>3</sub>CN (10 mL) and was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (120 mg, 0.4 mmol) and N,N-dimethylaminopyridine (DMAP, 110 mg, 0.88 mmol). After 4 h, more DMAP (0.55 g, 4.4 mmol) was added to the reaction mixture. After stirring for 1.5 h at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic phase was evaporated under reduced pressure. The residue was purified by chromatography on silica gel affording the crude carbamate 5a (220 mg) containing some p-nitrophenol. The crude 5a was repurified by HPLC (50% CH<sub>3</sub>CN /50% H<sub>2</sub>O) to afford pure carbamate 5a (176 mg, 46%, 2 steps). <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.20-7.36 (m, 1H), 6.94 (d, 1H), 5.64 (d, 1H), 5.10-5.25 (2 q(ab) overlap, 4H), 4.90-5.10 (m, 1H), 4.90 (d, 1H), 4.34 (d, 2H), 3.82-3.91 (m + s, 6H), 3.63-3.70 (m, 3H), 2.79-3.30 (m, 7H), 1.80-1.90 (m, 1H), 1.40-1.50 (m, 1H), 0.94 (d, 3H), 0.89 (d, 3H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): 17.2 ppm.

#### Example 16

Carbamate 5b: An ice-cold solution of crude amine 4b (89  $\mu$ mol) in CH<sub>3</sub>CN (5 mL) and was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (26mg, 89  $\mu$ mol) and N,N-dimethylaminopyridine (DMAP, 22 mg, 0.17 mmol). After 1 h at 0°C, more DMAP (10 mg, 82  $\mu$ mol) was added to the reaction mixture. After stirring for 2 h at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic phase was evaporated under reduced pressure. The residue was purified by HPLC (C18 column, 45% CH<sub>3</sub>CN/55% H<sub>2</sub>O) to afford pure carbamate 5b (18.8 mg, 29%, 3 steps). <sup>1</sup>H NMR (CDCl<sub>3</sub>):

$\delta$  7.38 (d, 2H), 7.20-7.36 (m, 6H), 7.0 (d, 1H), 5.64 (d, 1H), 4.96-5.03 (m, 2H), 4.39 (d, 2H), 4.20-4.31 (2q overlap, 4H) 3.80-4.00 ((s overlap with m, 7H), 3.60-3.73 (m, 2H), 3.64-3.70 (m, 2H), 2.85-3.30 (m, 7H), 1.80-1.95 (m, 1H), 1.55-1.75 (m, 1H), 1.35-1.50 (s overlap with m, 7H), 0.94 (d, 3H), 0.88 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 18.1 ppm.

#### Example 17

Phosphonic acid 6: A solution of dibenzylphosphonate 5a (50 mg, 58  $\mu\text{mol}$ ) was dissolved in MeOH (5 mL) and EtOAc (3 mL) and treated with 10% Pd/C (25 mg) and was stirred at room temperature under  $\text{H}_2$  atmosphere (balloon) for 8 h. The catalyst was filtered off. The filtrate was concentrated and redissolved in MeOH (5 mL), treated with 10% Pd/C (25 mg) and was stirred at room temperature under  $\text{H}_2$  atmosphere (balloon) overnight. The catalyst was filtered off. The filtrate was evaporated under reduced pressure to afford phosphonic acid 6 (38 mg, quantitatively).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.42 (m, 1H), 7.36 (s, 1H), 7.10-7.25 (m, 6H), 5.58 7 (d, 1H), 4.32 (d, 2H), 3.90 (s, 3H), 3.60-3.80 (m, 6H), 3.38 (d, 1H), 2.85-3.25 (m, 5H), 2.50-2.60 (m, 1H), 1.95-2.06 (m, 1H), 1.46-1.60 (m, 1H), 1.30-1.40 (m, 1H), 0.93(d, 3H), 0.89 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ ): 14.8 ppm; MS (ESI): 671 (M - H).

#### Example 18

Amine 7: To a  $0^\circ\text{C}$  cold solution of diethylphosphonate 3b (80 mg, 0.118 mmol) in  $\text{CH}_2\text{Cl}_2$  was treated with  $\text{BBr}_3$  in  $\text{CH}_2\text{Cl}_2$  (0.1 mL of 1 M solution, 1 mmol). The solution was stirred at  $0^\circ\text{C}$  10 min and then warmed to room temperature and stirred for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was redissolved in  $\text{CH}_2\text{Cl}_2$  (containing some  $\text{CH}_3\text{OH}$ , concentrated, azeotroped with  $\text{CH}_3\text{CN}$  three times. The crude amine 7 was used for next reaction without further purification.

#### Example 19

Carbamate 8: An ice-cold solution of crude amine 7 (0.118 mmol) in  $\text{CH}_3\text{CN}$  (5 mL) and was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-b]furan-2-yl 4-nitrophenyl carbonate (35 mg, 0.118 mmol) and N,N-dimethylaminopyridine (29 mg, 0.24mmol), warmed to room temperature. After stirring for 1 h at room temperature, more DMAP (20 mg, 0.16 mmol) was added to reaction mixture. After 2 h stirred at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was evaporated under reduced pressure. The residue

was purified by HPLC on C18 (CH<sub>3</sub>CN-55% H<sub>2</sub>O) to afford the desired carbamate 8 (11.4 mg, 13.4%) as an off-white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 7.20-7.40 (m, 7H), 7.00 (d, 1H), 5.64 (d, 1H), 5.00-5.31 (m, 2H), 4.35 (d, 2H), 4.19-4.30 (2q overlap, 4H), 3.80-4.00 (m, 4H), 3.68-3.74 (m, 2H), 3.08-3.20 (m, 3H), 2.75-3.00 (m, 4H), 1.80-1.90 (m, 1H), 1.55-1.75 (m, 1H), 1.38 (t, 6H), 0.91 (2d overlap, 6H). <sup>31</sup>P NMR (CD<sub>3</sub>OD): δ 19.5 ppm.

### Example Section K

#### Example 1

Monophenyl-monolactate 3: A mixture of monoacid 1 (0.500 g, 0.7 mmol), alcohol 2 (0.276 g, 2.09 mmol) and dicyclohexylcarbodiimide (0.431 g, 2.09 mmol) in dry pyridine (4 mL) was placed into a 70°C oil bath and heated for two hours. The reaction was monitored by TLC assay (SiO<sub>2</sub>, 70% ethyl acetate in hexanes as eluent, product R<sub>f</sub> = 0.68, visualization by UV). The reaction contents were cooled to ambient temperature with the aid of a cool bath and diluted with dichloromethane (25 mL). TLC assay may show presence of starting material. The diluted reaction mixture was filtered to remove solids. The filtrate was then cooled to 0°C and charged with 0.1 N HCl (10 mL). The pH 4 mixture was stirred for 10 minutes and poured into separatory funnel to allow the layers to separate. The lower organic layer was collected and dried over sodium sulfate. The drying agent was filtered off and the filtrate concentrated to an oil via rotary evaporator (< 30°C warm bath). The crude product oil was purified on pretreated silica gel (deactivated using 10% methanol in dichloromethane followed by rinse with 60% ethyl acetate in dichloromethane). The product was eluted with 60% ethyl acetate in dichloromethane to afford the product monophenyl-monolactate 3 as a white foam (0.497 g, 86% yield). <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.75 (d, 2H), 7.40-7.00 (m, 14H), 5.65 (d, 1H), 5.20-4.90 (m, 4H), 4.70 (d, 1H), 4.55-4.50 (m, 1H), 4.00-3.80 (m, 4H), 3.80-3.60 (m, 3H), 3.25-2.75 (m, 7H), 1.50 (d, 3H), 1.30-1.20 (m, 7H), 0.95 (d, 3H), 0.85 (d, 3H). <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 16.2, 13.9.

#### Example 2

Monophenyl-monoamidate 5: A mixture of monoacid 1 (0.500 g, 0.70 mmol), amine hydrochloride 4 (0.467 g, 2.78 mmol) and dicyclohexylcarbodiimide (0.862 g, 4.18 mmol) in dry pyridine (8 mL) was placed into a 60°C oil bath, and heated for one hour (at this temperature, product degrades if heating continues beyond this point). The reaction was

monitored by TLC assay (SiO<sub>2</sub>, 70% ethyl acetate in hexanes as eluent, product R<sub>f</sub> = 0.39, visualization by UV). The contents were cooled to ambient temperature and diluted with ethyl acetate (15 mL) to precipitate a white solid. The mixture was filtered to remove solids and the filtrate was concentrated via rotary evaporator to an oil. The oil was diluted with dichloromethane (20 mL) and washed with 0.1 N HCl (2 x 20 mL), water (1 x 20 mL) and dilute sodium bicarbonate (1 x 20 mL). The organic layer was dried over sodium sulfate, filtered, and concentrated to an oil via rotary evaporator. The crude product oil was dissolved in dichloromethane (10 mL). Hexane was slowly charged to the stirring solution until cloudiness persisted. The cloudy mixture was stirred for a few minutes until TLC assay showed that the dichloromethane/hexane layer contained no product. The dichloromethane/hexanes layer was decanted and the solid was further purified on silica gel first pretreated with 10% methanol in ethyl acetate and rinsed with 50% ethyl acetate in hexanes. The product 5 was eluted with 50% ethyl acetate in hexanes to afford a white foam (0.255 g, 44% yield) upon removal of solvents. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.75 (d, 2H), 7.40-7.15 (m, 10H), 7.15-7.00 (t, 2H), 5.65 (d, 1H), 5.10-4.90 (m, 3H), 4.50-4.35 (m, 2H), 4.25-4.10 (m, 1H), 4.00-3.60 (m, 8H), 3.20-2.75 (m, 7H), 1.40-1.20 (m, 11H), 0.95 (d, 3H), 0.85 (d, 3H). <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 19.1, 18.0.

### Example 3

Bisamidate 8: A solution of triphenylphosphine (1.71 g, 6.54 mmol) and aldrithiol (1.44 g, 6.54 mmol) in dry pyridine (5 mL), stirred for at least 20 minutes at room temperature, was charged into a solution of diacid 6 (1.20 g, 1.87 mmol) and amine hydrochloride 7 (1.30 g, 7.47 mmol) in dry pyridine (10 mL). Diisopropylethylamine (0.97 g, 7.48 mmol) was then added to this combined solution and the contents were stirred at room temperature for 20 hours. The reaction was monitored by TLC assay (SiO<sub>2</sub>, 5:5:1 ethyl acetate/hexanes/methanol as eluent, product R<sub>f</sub> = 0.29, visualization by UV). The reaction mixture was concentrated via rotary evaporator and dissolved in dichloromethane (50 mL). Brine (25 mL) was charged to wash the organic layer. The aqueous layer was back extracted with dichloromethane (1 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated via rotary evaporator to afford an oil. The crude product oil was purified on silica gel using 4% isopropanol in dichloromethane as eluent. The combined fractions containing the product may have residual amine contamination. If so, the fractions were concentrated via rotary evaporator and further purified by silica gel chromatography

using a gradient of 1:1 ethyl acetate/hexanes to 5:5:1 ethyl acetate/hexanes/methanol solution as eluent to afford the product 8 as a foam (0.500 g, 30% yield).

#### Example 4

- Diacid 6: A solution of dibenzylphosphonate 9 (8.0 g, 9.72 mmol) in ethanol (160 mL) and ethyl acetate (65 mL) under a nitrogen atmosphere and at room temperature was charged 10% Pd/C (1.60 g, 20 wt%). The mixture was stirred and evacuated by vacuum and purged with hydrogen several times. The contents were then placed under atmospheric pressure of hydrogen via a balloon. The reaction was monitored by TLC assay (SiO<sub>2</sub>, 7:2.5:0.5 dichloromethane/methanol/ammonium hydroxide as eluent, product R<sub>f</sub> = 0.05, visualization by UV) and was judged complete in 4 to 5 hours. The reaction mixture was filtered through a pad of celite to remove Pd/C and the filter cake rinsed with ethanol/ethyl acetate mixture (50 mL). The filtrate was concentrated via rotary evaporation followed by several co-evaporations using ethyl acetate (3 x 50 mL) to remove ethanol. The semi-solid diacid 6, free of ethanol, was carried forward to the next step without purification.

#### Example 5

Diphenylphosphonate 10: To a solution of diacid 6 (5.6 g, 8.71 mmol) in pyridine (58 mL) at room temperature was charged phenol (5.95 g, 63.1 mmol). To this mixture, while stirring, was charged dicyclohexylcarbodiimide (7.45 g, 36.0 mmol). The resulting cloudy, yellow mixture was placed in a 70-80°C oil bath. The reaction was monitored by TLC assay (SiO<sub>2</sub>, 7:2.5:0.5 dichloromethane/methanol/ammonium hydroxide as eluent, diacid R<sub>f</sub> = 0.05, visualization by UV for the disappearance of starting material. SiO<sub>2</sub>, 60% ethyl acetate in hexanes as eluent, diphenyl R<sub>f</sub> = 0.40, visualization by UV) and was judged complete in 2 hours. To the reaction mixture was charged isopropyl acetate (60 mL) to produce a white precipitation. The slurry was filtered through a pad of celite to remove the white precipitate and the filter cake rinsed with isopropyl acetate (25 mL). The filtrate was concentrated via rotary evaporator. To the resulting yellow oil was charged a premixed solution of water (58 mL) and 1N HCl (55 mL) followed by isopropyl acetate (145 mL). The mixture was stirred for one hour in an ice bath. After separating the layers, the aqueous layer was back extracted with ethyl acetate (2 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated via rotary evaporator. The crude product oil was purified by silica gel column chromatography using 50% ethyl acetate in hexanes as eluent to afford the

product 10 as a white foam (3.52 g, 51% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75 (d, 2H), 7.40-7.20 (m, 15H), 7.10 (d, 2H), 5.65 (d, 1H), 5.10-4.90 (m, 2H), 4.65 (d, 2H), 4.00-3.80 (m, 4H), 3.75-3.65 (m, 3H), 3.25-2.75 (m, 7H), 1.90-1.75 (m, 1H), 1.70-1.60 (m, 1H), 1.50-1.40 (m, 1H), 0.90 (d, 3H), 0.85 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.9.

#### Example 6

Monophenyl 1: To a solution of diphenyl 10 (3.40 g, 4.28 mmol) in acetonitrile (170 mL) at 0°C was charged 1N sodium hydroxide (4.28 mL). The reaction was monitored by TLC assay ( $\text{SiO}_2$ , 7:2.5:0.5 dichloromethane/methanol/ammonium hydroxide as eluent, diphenyl  $R_f$  = 0.65, visualization by UV for the disappearance of starting material. Product monophenyl  $R_f$  = 0.80, visualization by UV). Additional 1N NaOH was added (if necessary) until the reaction was judged complete. To the reaction contents at 0°C was charged Dowex  $\text{H}^+$  (Dowex 50WX8-200) (4.42 g) and stirred for 30 minutes at which time the pH of the mixture reached pH 1 (monitored by pH paper). The mixture was filtered to remove the Dowex resin and the filtrate was concentrated via rotary evaporation (water bath < 40°C). The resulting solution was co-evaporated with toluene to remove water (3 x 50 mL). The white foam was dissolved in ethyl acetate (8 mL) followed by slow addition of hexanes (16 mL) over 30 minutes to induce precipitation. A premixed solution of 2:1 hexanes/ethyl acetate solution (39 mL) was charged to the precipitated material and stirred. The product 1 was filtered and rinsed with premixed solution of 2:1 hexanes/ethyl acetate solution (75 mL) and dried under vacuum to afford a white powder (2.84 g, 92% yield).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.80 (d, 2H), 7.40-7.30 (m, 2H), 7.20-7.15 (m, 11H), 5.55 (d, 1H), 4.50 (d, 2H), 3.95-3.85 (m, 1H), 3.80-3.60 (m, 5H), 3.45 (bd, 1H), 3.25-3.15 (m, 2H), 3.00-2.80 (m, 3H), 2.60-2.45 (m, 1H), 2.10-1.95 (m, 2H), 1.85-1.60 (m, 2H), 1.50-1.40 (m, 1H), 1.40-1.30 (m, 1H), 0.95 (d, 3H), 0.85 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  13.8. The monophenyl product 1 is sensitive to silica gel. On contact with silica gel 1 converts to an unknown compound possessing  $^{31}\text{P}$  NMR chemical shift of 8 ppm. However, the desired monophenyl product 1 can be regenerated by treatment of the unknown compound with 2.5 M NaOH in acetonitrile at 0°C for one hour followed by Dowex  $\text{H}^+$  treatment as described above.

#### Example 7

Dibenzylphosphonate 9: To a solution of phenol 11 (6.45 g, 11.8 mmol) in tetrahydrofuran (161 mL) at room temperature was charged triflate reagent 12 (6.48 g, 15.3 mmol). Cesium



carbonate (11.5 g, 35.3 mmol) was added and the mixture was stirred and monitored by TLC assay ( $\text{SiO}_2$ , 5% methanol in dichloromethane as eluent, dibenzyl product  $R_f = 0.26$ , visualization by UV or ninhydrin stain and heat). Additional  $\text{Cs}_2\text{CO}_3$  was added until the reaction was judged complete. To the reaction contents was charged water (160 mL) and the mixture extracted with ethyl acetate (2 x 160 mL). The combined organic layer was dried over sodium sulfate, filtered, and concentrated via rotary evaporator to afford a viscous oil. The crude oil was purified by silica gel column chromatography using a gradient of 100% dichloromethane to 1% methanol in dichloromethane to afford product 9 as a white foam (8.68 g, 90% yield).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75 (d, 2H), 7.40-7.20 (m, 16H), 6.95 (d, 2H), 5.65 (d, 1H), 5.20-4.90 (m, 6H), 4.25 (d, 2H), 4.00-3.80 (m, 4H), 3.75-3.65 (m, 3H), 3.20-2.75 (m, 7H), 1.90-1.75 (m, 1H), 1.30-1.20 (m, 1H), 0.90 (d, 3H), 0.85 (d, 3H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.1.

#### Example 7a

Hydroxyphenylsulfonamide 14: To a solution of methoxyphenylsulfonamide 13 (35.9 g, 70.8 mmol) in dichloromethane (3.5 L) at  $0^\circ\text{C}$  was charged boron tribromide (1M in DCM, 40.1 mL, 425 mmol). The reaction content was allowed to warm to room temperature, stirred over two hours, and monitored by TLC assay ( $\text{SiO}_2$ , 10% methanol in dichloromethane as eluent, dibenzyl product  $R_f = 0.16$ , visualization by UV). To the contents at  $0^\circ\text{C}$  was slowly charged propylene oxide (82 g, 1.42 mmol). Methanol (200 mL) was added and the reaction mixture was concentrated via rotary evaporator to afford a viscous oil. The crude product mixture was purified by silica gel column chromatography using 10% methanol in dichloromethane to afford the product 14 as a foam (22 g, 80% yield).  $^1\text{H}$  NMR (DMSO)  $\delta$  7.60 (d, 2H), 7.30-7.20 (m, 5H), 6.95 (d, 2H), 3.90-3.75 (m, 1H), 3.45-3.20 (m, 5H), 3.00-2.55 (m, 5H), 2.50-2.40 (m, 1H), 1.95-1.85 (m, 1H), 0.85 (d, 3H), 0.80 (d, 3H).

#### Example 8

Cisfuran carbamate 16: To a solution of amine 14 (20.4 g, 52.0 mmol) in acetonitrile (600 mL) at room temperature was charged dimethylaminopyridine (13.4 g, 109 mmol) followed by cisfuran *p*-nitrophenylcarbonate reagent 15 (14.6 g, 49.5 mmol). The resulting solution was stirred at room temperature for at least 48 hours and monitored by TLC assay ( $\text{SiO}_2$ , 10% methanol in dichloromethane as eluent, cisfuran product  $R_f = 0.34$ , visualization by UV). The reaction mixture was concentrated via rotary evaporator. The crude product mixture was

purified by silica gel column chromatography using a gradient of 60% ethyl acetate in hexanes to 70% ethyl acetate in hexanes to afford the product 16 as a solid (18.2 g, 64% yield).  $^1\text{H}$  NMR (DMSO)  $\delta$  10.4 (bs, 1H), 7.60 (d, 2H), 7.30-7.10 (m, 6H), 6.95 (d, 2H), 5.50 (d, 1H), 4.85 (m, 1H), 3.85 (m, 1H), 3.70 (m, 1H), 3.65-3.50 (m, 4H), 3.30 (d, 1H), 3.05-2.95 (m, 2H), 2.80-2.65 (m, 3H), 2.50-2.40 (m, 1H), 2.00-1.90 (m, 1H), 1.45-1.20 (m, 2H), 0.85 (d, 3H), 0.80 (d, 3H).

### Example Section L

#### Example 1

Monobenzyl phosphonate 2 A solution of dibenzylphosphonate 1 (150 mg, 0.175 mmol) was dissolved in toluene (1 mL), treated with DABCO (20 mg, 0.178 mmol) and was refluxed under  $\text{N}_2$  atmosphere (balloon) for 3 h. The solvent was removed and the residual was dissolved in aqueous HCl (5%). The aqueous layer was extracted with ethyl acetate and the organic layer was dried over sodium sulfate. After evaporation to yield the monobenzyl phosphonate 2 (107 mg, 80%) as a white powder.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.75 (d,  $J = 5.4$  Hz, 2H), 7.42-7.31 (m, 5H) 7.16 (d,  $J = 5.4$  Hz, 2H), 7.01 (d,  $J = 5.4$  Hz, 2H), 6.86 (d,  $J = 5.4$  Hz, 2H), 5.55 (d,  $J = 3.3$  Hz, 1H), 5.14 (d,  $J = 5.1$  Hz, 2H), 4.91 (m, 1H), 4.24-3.66 (m overlapping s, 11H), 3.45 (m, 2H), 3.14-2.82 (m, 6H), 2.49 (m, 1H), 2.01 (m, 1H), 1.51-1.34 (m, 2H), 0.92 (d,  $J = 3.9$  Hz, 3H), 0.87 (d,  $J = 3.9$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  20.5; MS (ESI) 761 (M-H).

#### Example 2

Monobenzyl, ethyl phosphonate 3 To a solution of monobenzyl phosphonate 2 (100 mg, 0.13 mmol) in dry THF (5 mL) at room temperature under  $\text{N}_2$  was added  $\text{Ph}_3\text{P}$  (136 mg, 0.52 mmol) and ethanol (30  $\mu\text{L}$ , 0.52 mmol). After cooled to  $0^\circ\text{C}$ , DEAD (78  $\mu\text{L}$ , 0.52 mmol) was added. The mixture was stirred for 20 h at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by using chromatograph on silica gel (10% to 30% ethyl acetate / hexane) to afford the monobenzyl, ethyl phosphonate 3 (66 mg, 64%) as white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 7.70 (d,  $J = 8.7$  Hz, 2H), 7.43-7.34 (m, 5H) 7.14 (d,  $J = 8.4$  Hz, 2H), 7.01 (d,  $J = 8.7$  Hz, 2H), 6.84 (d,  $J = 8.4$  Hz, 2H), 5.56 (d,  $J = 5.4$  Hz, 1H), 5.19 (d,  $J = 8.7$  Hz, 2H), 5.00 (m, 2H), 4.22-3.67 (m overlapping s, 13H), 3.18-2.76 (m, 7H), 1.82-1.54 (m, 3H), 1.33 (t,  $J = 7.0$  Hz, 3H), 0.92 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.8; MS (ESI) 813 (M+Na).

### Example 3

**Monoethyl phosphonate 4** A solution of monobenzyl, ethyl phosphonate 3 (60 mg) was dissolved in EtOAc (2 mL), treated with 10% Pd/C (6 mg) and was stirred under H<sub>2</sub> atmosphere (balloon) for 2h. The catalyst was removed by filtration through celite. The filtered was evaporated under reduced pressure, the residue was triturated with ether and the solid was collected by filtration to afford the monoethyl phosphonate 4 (50 mg, 94%) as white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) 7.76 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.7Hz, 2H), 6.89 (d, J = 8.4 Hz, 2H), 5.58 (d, J = 5.4 Hz, 1H), 5.90 (m, 1H), 4.22-3.67 (m overlapping s, 13H), 3.18-2.50 (m, 7H), 1.98(m, 1H), 1.56 (m, 2H), 1.33 (t, J = 6.9 Hz, 3H), 0.92 (d, J = 6.6Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 18.7; MS (ESI) 700 (M-H).

### Example 4

**Monophenyl, ethyl phosphonate 5** To a solution of phosphonic acid 11 (800 mg, 1.19 mmol) and phenol (1.12 g, 11.9 mmol) in pyridine (8 mL) was added ethanol (69 µL, 1.19 mmol) and 1, 3-dicyclohexylcarbodiimide (1g, 4.8 mmol). The solution was stirred at 70°C for 2h. The reaction mixture was cooled to room temperature, then diluted with ethyl acetate (10 mL) and filtered. The filtrate was evaporated under reduced pressure to remove pyridine. The residue was dissolved in ethyl acetate and the organic phase was separated and washed with brine, dried over MgSO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel to give monophenyl, ethyl phosphonate 5 (600 mg, 65%) as white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.72 (d, J = 9 Hz, 2H), 7.36-7.18 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 6.98 (d, J = 9Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.00 (m, 2H), 4.34 (m, 4H), 3.94-3.67 (m overlapping s, 9H), 3.18-2.77 (m, 7H), 1.82-1.54 (m, 3H), 1.36 (t, J = 7.2 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 16.1; MS (ESI) 799 (M+Na).

### Example 5

**Sulfonamide 6** To a suspension of epoxide 5 (3 g, 8.12 mmol) in 2-propanol (30 mL) was added isobutylamine (8 mL, 81.2 mmol) and the solution was stirred at 80°C for 1 h. The solution was evaporated under reduced pressure and the crude solid was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) and cooled to 0°C. TEA (2.3 mL, 16.3mmol) was added followed by the addition of 4-nitrobenzenesulfonyl chloride (1.8 g, 8.13 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and the solution was

stirred for 30 min at 0°C, warmed to room temperature and evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated under reduced pressure. The crude product was recrystallized from EtOAc/hexane to give the sulfonamide 6 (4.6 g, 91%) as an off-white solid. MS (ESI) 650 (M+Na).

#### Example 6

**Phenol 7** A solution of sulfonamide 6 (4.5 g, 7.1 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at 0°C was treated with BBr<sub>3</sub> (1M in CH<sub>2</sub>Cl<sub>2</sub>, 50mL). The solution was stirred at 0°C to room temperature for 48h. CH<sub>3</sub>OH (10 mL) was carefully added. The solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and saturated NaHCO<sub>3</sub>. The organic phase washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (10% - MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the phenol 7 (2.5 g, 80%) as an off-white solid. MS (ESI) 528 (M+H).

#### Example 7

**Carbamate 8** A solution of sulfonamide 7 (2.5 g, 5.7 mmol) in CH<sub>3</sub>CN (100 mL) and was treated with proton-sponge (3 g, 14 mmol) and followed by (3R, 3aR, 6aS)-hexahydrofuro[2,3-*b*]furan-2-yl 4-nitrophenyl carbonate (1.7 g, 5.7 mmol) at 0°C. After stirring for 48h at room temperature, the reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 10% HCl. The organic phase was washed with saturated NaCl, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (10% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) affording the carbamate 8 (2.1g, 62 %) as a white solid. MS (ESI) 616 (M+Na).

#### Example 8

**Diethylphosphonate 9** To a solution of carbamate 8 (2.1 g, 3.5 mmol) in CH<sub>3</sub>CN (50 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (3.2 g, 9.8 mmol) and diethyltriflate (1.6g, 5.3 mmol). The mixture was stirred at room temperature for 1h. After removed the solvent, the residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was chromatographed on silica gel (1% to 5% MeOH /CH<sub>2</sub>Cl<sub>2</sub>) to afford the diethylphosphonate 9 as a white solid: <sup>1</sup>H NMR

(CDCl<sub>3</sub>)  $\delta$  8.35 (d, J = 9 Hz, 2H), 7.96 (d, J = 9 Hz, 2H), 7.13 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.4 Hz, 2H), 5.63 (d, J = 5.1 Hz, 1H), 5.18-5.01 (m, 2H), 4.27-4.17 (m, 6H), 3.94-3.67 (m, 7H), 3.20-2.73 (m, 7H), 1.92-1.51 (m, 3H), 1.35 (t, J = 7.2 Hz, 6H), 0.88-0.85 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>)  $\delta$  19.2; MS (ESI) 756 (M+Na).

#### Example 9

**Amine 10** A solution of diethylphosphonate 9 (1 g) was dissolved in EtOH (100 mL), treated with 10% Pd/C (300 mg) and was stirred under H<sub>2</sub> atmosphere (balloon) for 3h. The reaction was purged with N<sub>2</sub>, and the catalyst was removed by filtration through celite. After evaporation of the filtrate, the residue was triturated with ether and the solid was collected by filtration to afford the amine 10 (920 mg, 96%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.41 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 5.67 (d, J = 5.1 Hz, 1H), 5.13-5.05 (m, 2H), 4.42 (s, 2H), 4.29-4.20 (m, 6H), 4.00-3.69 (m, 7H), 3.00-2.66 (m, 7H), 1.80-1.69 (m, 3H), 1.38 (m, 6H), 0.94 (d, J = 6.4 Hz, 3H), 0.86 (d, J = 6.4 Hz, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>)  $\delta$  19.4; MS (ESI) 736 (M+Na).

| Compound | R <sub>1</sub> | R <sub>2</sub> |
|----------|----------------|----------------|
| 16a      | Gly-Et         | Gly-Et         |
| 16b      | Gly-Bu         | Gly-Bu         |
| 16j      | Phe-Bu         | Phe-Bu         |
| 16k      | NHEt           | NHEt           |

#### Example 10

**Synthesis of Bisamidates 16a.** A solution of phosphonic acid 11 (100 mg, 0.15 mmol) L-alanine ethyl ester hydrochloride (84 mg, 0.6 mmol) was dissolved in pyridine (5 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of Ph<sub>3</sub>P (118 mg, 0.45 mmol) and 2,2'-dipyridyl disulfide (99 mg, 0.45 mmol) in pyridine (1 mL) stirring for 20h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>). The purified product was suspended in ether and was evaporated under reduced pressure to afford bisamidate 16a (90 mg, 72%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.72 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.68 (d, J = 5.1 Hz, 1H), 5.05 (m, 1H), 4.25 (d, J = 9.9 Hz, 2H), 4.19 (q, 4H),

3.99-3.65 (m overlapping s, 13H), 3.41 (m, 1H), 3.20-2.81 (m, 7H), 1.85-1.60 (m, 3H), 1.27 (t,  $J = 7.2$  Hz, 6H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.89 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  21.8; MS (ESI) 843 (M+H).

#### Example 11

Synthesis of Bisamidates 16b. A solution of phosphonic acid 11 (100 mg, 0.15 mmol) L-alanine n-butyl ester hydrochloride (101 mg, 0.6 mmol) was dissolved in pyridine (5 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of  $\text{Ph}_3\text{P}$  (118 mg, 0.45 mmol) and 2,2'-dipyridyl disulfide (99 mg, 0.45 mmol) in pyridine (1 mL) stirring for 20h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ). The purified product was suspended in ether and was evaporated under reduced pressure to afford bisamidate 16b (100 mg, 74%) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 9$  Hz, 2H), 7.15 (d,  $J = 9$  Hz, 2H), 7.01 (d,  $J = 9$  Hz, 2H), 6.87 (d,  $J = 9$  Hz, 2H), 5.67 (d,  $J = 5.4$  Hz, 1H), 5.05 (m, 1H), 4.96 (m, 1H), 4.25 (d,  $J = 9.9$  Hz, 2H), 4.11 (t,  $J = 6.9$  Hz, 4H), 3.99-3.71 (m overlapping s, 13H), 3.41 (m, 1H), 3.20-2.80 (m, 7H), 1.87-1.60 (m, 7H), 1.42 (m, 4H), 0.96-0.88 (m, 12H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  21.8; MS (ESI) 890 (M+H).

#### Example 12

Synthesis of Bisamidates 16j. A solution of phosphonic acid 11 (100 mg, 0.15 mmol) L-phenylalanine n-butyl ester hydrochloride (155 mg, 0.6 mmol) was dissolved in pyridine (5 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of  $\text{Ph}_3\text{P}$  (118 mg, 0.45 mmol) and 2,2'-dipyridyl disulfide (99 mg, 0.45 mmol) in pyridine (1 mL) stirring for 36h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ). The purified product was suspended in ether and was evaporated under reduced pressure to afford bisamidate 16j (106 mg, 66%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.31-7.10 (m, 12H), 7.01 (d,  $J = 9$  Hz, 2H), 6.72 (d,  $J = 8.7$  Hz, 2H), 5.67 (d,  $J = 5.1$  Hz, 1H), 5.05 (m, 1H), 4.96 (m, 1H), 4.35-3.98 (m, 7H), 3.90-3.61 (m overlapping s, 10H), 3.19-2.78 (m, 11H), 1.87-1.25 (m, 11H), 0.96-0.88 (m, 12H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.3; MS (ESI) 1080 (M+H).

Example 13

Synthesis of Bisamidates 16k. A solution of phosphonic acid 11 (80 mg, 0.12 mmol), ethylamine (0.3 mL, 2M in THF, 0.6 mmol) was dissolved in pyridine (5 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of Ph<sub>3</sub>P (109 mg, 0.42 mmol) and 2,2'-dipyridyl disulfide (93 mg, 0.42 mmol) in pyridine (1 mL) stirring for 48h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>). The purified product was suspended in ether and was evaporated under reduced pressure to afford bisamidate 16k (60 mg, 70%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.15 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 5.67 (d, J = 5.1 Hz, 1H), 5.05-4.95 (m, 2H), 4.15 (d, J = 9.6 Hz, 2H), 3.99-3.72 (m overlapping s, 9H), 3.18-2.81 (m, 11H), 2.55 (br, 1H), 1.85-1.65 (m, 3H), 1.18 (t, J = 7.2 Hz, 6H), 0.93 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 21.6; MS (ESI) 749 (M+Na).

| Compound | R <sub>1</sub> | R <sub>2</sub> |
|----------|----------------|----------------|
| 30a      | OPh            | Ala-Me         |
| 30b      | OPh            | Ala-Et         |
| 30c      | OPh            | (D)-Ala-iPr    |
| 30d      | OPh            | Ala-Bu         |
| 30e      | OBn            | Ala-Et         |

Example 14

Monoamidate 30a (R<sub>1</sub> = OPh, R<sub>2</sub> = Ala-Me) To a flask was charged with monophenyl phosphonate 29 (75 mg, 0.1 mmol), L-alanine methyl ester hydrochloride (4.0 g, 22 mmol) and 1,3-dicyclohexylcarbodiimide (84 mg, 0.6 mmol), then pyridine (1 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60 – 70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was partitioned between ethyl acetate and HCl (0.2 N), the ethyl acetate phase was washed with water and NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate/hexane 1:5) to give 30a (25 mg, 30%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.73-7.24 (m, 5H) 7.19-7.15 (m, 2H), 7.01 (d, J = 8.7 Hz, 2H), 6.90-6.83 (m, 2H), 5.65 (d, J = 5.1 Hz, 1H), 5.01 (m, 2H), 4.30 (m, 2H), 3.97-3.51 (m overlapping s, 12H), 3.20-2.77 (m, 7H), 1.81 (m, 1H), 1.58

(m, 3H), 0.92 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4 and 19.3; MS (ESI) 856 (M+Na).

#### Example 15

Monoamidate 30b ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = \text{Ala-Et}$ ) was synthesized in the same manner in 35% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.24 (m, 5H) 7.19-7.15 (m, 2H), 7.01 (d,  $J = 8.7$  Hz, 2H), 6.90-6.83 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.01 (m, 3H), 4.30 -3.67 (m overlapping s, 14H), 3.18-2.77 (m, 7H), 1.81-1.35 (m, 6H), 1.22 (m, 3H), 0.92 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4 and 19.3; MS (ESI) 870 (M+Na).

#### Example 16

Monoamidate 30c ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = (\text{D})\text{-Ala-iPr}$ ) was synthesized in the same manner in 52% yield. Isomer A  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.24 (m, 5H) 7.19-7.15 (m, 2H), 7.01 (d,  $J = 8.7$  Hz, 2H), 6.90-6.83 (m, 2H), 5.66 (m, 1H), 5.01 (m, 3H), 4.30 -3.67 (m overlapping s, 14H), 3.18-2.77 (m, 7H), 1.81-1.35 (m, 6H), 1.23 (m, 6H), 0.92 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4; MS (ESI) 884 (M+Na). Isomer B  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.24 (m, 5H) 7.19-7.15 (m, 2H), 7.01 (d,  $J = 8.7$  Hz, 2H), 6.90-6.83 (m, 2H), 5.66 (m, 1H), 5.01 (m, 3H), 4.30 -3.67 (m overlapping s, 14H), 3.18-2.77 (m, 7H), 1.81-1.35 (m, 6H), 1.23 (m, 6H), 0.92 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.3; MS (ESI) 884 (M+Na).

#### Example 17

Monoamidate 30d ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = \text{Ala-Bu}$ ) was synthesized in the same manner in 25% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.24 (m, 5H) 7.19-7.15 (m, 2H), 7.01 (d,  $J = 8.7$  Hz, 2H), 6.90-6.83 (m, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.01 (m, 3H), 4.30 -3.67 (m overlapping s, 16H), 3.18-2.77 (m, 7H), 1.81-1.35 (m, 8H), 1.22 (m, 3H), 0.92 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4 and 19.4; MS (ESI) 898 (M+Na).

#### Example 18

Monoamidate 30e ( $\text{R1} = \text{OBn}$ ,  $\text{R2} = \text{Ala-Et}$ ) To a flask was charged with monobenzyl phosphonate 2 (76 mg, 0.1 mmol), L-alanine methyl ester hydrochloride (4.0 g, 22 mmol) and 1, 3-dicyclohexylcarbodiimide (84 mg, 0.6 mmol), then pyridine (1 mL) was added under



N<sub>2</sub>. The resulted mixture was stirred at 60 – 70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was partitioned between ethyl acetate and HCl (0.2 N), the ethyl acetate phase was washed with water and NaHCO<sub>3</sub>, dried over Na<sub>2</sub>SO<sub>4</sub> filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate / hexane 1:5) to give 30a (25 mg, 30%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.38-7.34 (m, 5H), 7.13 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.86-6.80 (m, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.15-5.01 (m, 5H), 4.30 -3.67 (m overlapping s, 14H), 3.18-2.77 (m, 7H), 1.81-1.35 (m, 6H), 1.22 (m, 3H), 0.92 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 23.3 and 22.4; MS (ESI) 884 (M+Na).

| Compound | R <sub>1</sub> | R <sub>2</sub> |
|----------|----------------|----------------|
| 31a      | OPh            | Lac-iPr        |
| 31b      | OPh            | Lac-Et         |
| 31c      | OPh            | Lac-Bu         |
| 31d      | OPh            | (R)-Lac-Me     |
| 31e      | OPh            | (R)-Lac-Et     |

#### Example 19

Monolactate 31a (R<sub>1</sub> = OPh, R<sub>2</sub> = Lac-iPr): To a flask was charged with monophenyl phosphonate 29 (1.5 g, 2 mmol), isopropyl-(s)-lactate (0.88 mL, 6.6 mmol) and 1, 3-dicyclohexylcarbodiimide (1.36 g, 6.6 mmol), then pyridine (15 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60 – 70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was washed with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (ethyl acetate / CH<sub>2</sub>Cl<sub>2</sub> 1:5) to give 31a (1.39g, 81%) as a white solid. Isomer A <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.73-7.19 (m, 5H), 7.15 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.4 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.15-5.00 (m, 4H), 4.56-4.44 (m, 2H), 3.96 -3.68 (m overlapping s, 9H), 3.13-2.78 (m, 7H), 1.81-1.23 (m, 6H), 1.22 (m, 6H), 0.92 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.4; MS (ESI) 885 (M+Na). Isomer B <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.73-7.19 (m, 5H), 7.14 (d, J = 8.4 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.4 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.15-5.00 (m, 4H), 4.53 -4.41 (m, 2H), 3.96 -3.68

(m overlapping s, 9H), 3.13-2.78 (m, 7H), 1.81-1.23 (m, 6H), 1.22 (m, 6H), 0.92 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.3; MS (ESI) 885 ( $\text{M}+\text{Na}$ ).

#### Example 20

- i Monolactate 31b ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = \text{Lac-Et}$ ) was synthesized in the same manner in 75% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.14 (m, 7H), 6.99 (d,  $J = 8.7$  Hz, 2H), 6.88 (d,  $J = 8.7$  Hz, 2H), 5.63 (m, 1H), 5.19-4.95 (m, 3H), 4.44-4.40 (m, 2H), 4.17-4.12 (m, 2H), 3.95-3.67 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.58 (m, 6H), 1.23 (m, 3H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.5 and 15.4; MS (ESI) 872 ( $\text{M}+\text{Na}$ ).

#### Example 21

Monolactate 31c ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = \text{Lac-Bu}$ ) was synthesized in the same manner in 58% yield. Isomer A  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.19 (m, 5H), 7.14 (d,  $J = 8.4$  Hz, 2H), 7.00 (d,  $J = 8.7$  Hz, 2H), 6.90 (d,  $J = 8.4$  Hz, 2H), 5.63 (d,  $J = 5.4$  Hz, 1H), 5.15-5.00 (m, 3H), 4.56-4.51 (m, 2H), 4.17-4.10 (m, 2H), 3.95-3.67 (m overlapping s, 9H), 3.10-2.77 (m, 7H), 1.81-1.23 (m, 10H), 1.23 (m, 6H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3; MS (ESI) 899 ( $\text{M}+\text{Na}$ ). Isomer B  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.19 (m, 5H), 7.14 (d,  $J = 8.4$  Hz, 2H), 7.00 (d,  $J = 8.7$  Hz, 2H), 6.90 (d,  $J = 8.4$  Hz, 2H), 5.64 (d,  $J = 5.4$  Hz, 1H), 5.15-5.00 (m, 3H), 4.44-4.39 (m, 2H), 4.17-4.10 (m, 2H), 3.95-3.67 (m overlapping s, 9H), 3.10-2.77 (m, 7H), 1.81-1.23 (m, 10H), 1.23 (m, 6H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.87 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.3; MS (ESI) 899 ( $\text{M}+\text{Na}$ ).

#### Example 22

Monolactate 31d ( $\text{R1} = \text{OPh}$ ,  $\text{R2} = (\text{R})\text{-Lac-Me}$ ): To a stirred solution of monophenyl phosphonate 29 (100 mg, 0.13 mmol) in 10 mL of THF at room temperature under  $\text{N}_2$  was added methyl-(S)-lactate (54 mg, 0.52 mmol) and  $\text{Ph}_3\text{P}$  (136 mg, 0.52 mmol), followed by DEAD (82  $\mu\text{L}$ , 0.52 mmol). After 2 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate / hexane 1:1) to give 31d (33 mg, 30%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.73-7.14 (m, 7H), 6.99 (d,  $J = 8.7$  Hz, 2H), 6.88 (d,  $J = 8.7$  Hz, 2H), 5.63 (m, 1H),

5.19-4.95 (m, 3H), 4.44-4.40 (m, 2H), 3.95 -3.64 (m overlapping s, 12H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 4H), 0.91 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4 and 15.3; MS (ESI) 857 (M+Na).

#### Example 23

Monolactate 31e (R1 = OPh, R2 = (R)-Lac-Et): To a stirred solution of monophenyl phosphonate 29 (50 mg, 0.065 mmol) in 2.5 mL of THF at room temperature under  $\text{N}_2$  was added ethyl-(s)-lactate (31 mg, 0.52 mmol) and  $\text{Ph}_3\text{P}$  (68 mg, 0.26 mmol), followed by DEAD (41  $\mu\text{L}$ , 0.52 mmol). After 2 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate / hexane 1:1) to give 31e (28 mg, 50%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d, J = 8.7 Hz, 2H), 7.73-7.14 (m, 7H), 6.99 (d, J = 8.7 Hz, 2H), 6.85 (m, 2H), 5.63 (m, 1H), 5.19-4.95 (m, 3H), 4.44-4.40 (m, 2H), 4.17-4.12 (m, 2H), 3.95 -3.67 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.58 (m, 6H), 1.23 (m, 3H), 0.91 (d, J = 6.6 Hz, 3H), 0.87 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.5 and 15.4; MS (ESI) 872 (M+Na).

#### Example 24

Monolactate 32 (R1 = OBn, R2 = (S)-Lac-Bn): To a stirred solution of monobenzyl phosphonate 2 (76 mg, 0.1 mmol) in 0.5 mL of DMF at room temperature under  $\text{N}_2$  was added benzyl-(s)-lactate (27 mg, 0.15 mmol) and PyBOP (78 mg, 0.15 mmol), followed by DIEA (70  $\mu\text{L}$ , 0.4 mmol). After 3 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate / hexane 1:1) to give 32 (46 mg, 50%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d, J = 8.7 Hz, 2H), 7.38-7.44 (m, 10H), 7.13 (d, J = 8.4 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.81 (m, 2H), 5.63 (d, J = 5.1 Hz, 1H), 5.23-4.92 (m, 7H), 4.44-4.22 (m, 2H), 3.96 -3.67 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.58 (m, 6H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.8 and 19.6; MS (ESI) 947 (M+Na).

#### Example 25

Monolactate 33 (R1 = OBn, R2 = (R)-Lac-Bn): To a stirred solution of monobenzyl phosphonate 2 (76 mg, 0.1 mmol) in 5 mL of THF at room temperature under  $\text{N}_2$  was added benzyl-(s)-lactate (72 mg, 0.4 mmol) and  $\text{Ph}_3\text{P}$  (105 mg, 0.4 mmol), followed by DEAD

(60  $\mu$ L, 0.4 mmol). After 20 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate / hexane 1:1) to give 33 (44 mg, 45%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J$  = 8.7 Hz, 2H), 7.38-7.44 (m, 10H), 7.13 (m, 2H), 6.99 (d,  $J$  = 8.7 Hz, 2H), 6.81 (m, 2H), 5.63 (m, 1H), 5.23-4.92 (m, 7H), 4.44-2.2 (m, 2H), 3.96-3.67 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.58 (m, 6H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.8 and 19.6; MS (ESI) 947 (M+Na).

#### Example 26

- Monophosphonic acid 34: A solution of monobenzyllactate 32 (20 mg) was dissolved in EtOH/ EtOAc (3 mL/1 mL), treated with 10% Pd/C (4 mg) and was stirred under  $\text{H}_2$  atmosphere (balloon) for 1.5 h. The catalyst was removed by filtration through celite. The filtered was evaporated under reduced pressure, the residue was triturated with ether and the solid was collected by filtration to afford the monophosphonic acid 33 (15 mg, 94%) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.76 (d,  $J$  = 8.7 Hz, 2H), 7.18 (d,  $J$  = 8.7 Hz, 2H), 7.08 (d,  $J$  = 8.7 Hz, 2H), 6.90 (d,  $J$  = 8.7 Hz, 2H), 5.69 (d,  $J$  = 5.7 Hz, 1H), 5.03-4.95 (m, 2H), 4.20 (m, 2H), 3.90-3.65 (m overlapping s, 9H), 3.41 (m, 2H), 3.18-2.78 (m, 5H), 2.44 (m, 1H), 2.00 (m, 1H), 1.61-1.38 (m, 5H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  18.0; MS (ESI) 767 (M+Na).

#### Example 27

- Monophosphonic acid 35: A solution of monobenzyllactate 33 (20 mg) was dissolved in EtOH (3 mL), treated with 10% Pd/C (4 mg) and was stirred under  $\text{H}_2$  atmosphere (balloon) for 1h. The catalyst was removed by filtration through celite. The filtered was evaporated under reduced pressure, the residue was triturated with ether and the solid was collected by filtration to afford the monophosphonic acid 35 (15 mg, 94%) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.76 (d,  $J$  = 8.7 Hz, 2H), 7.18 (d,  $J$  = 8.7 Hz, 2H), 7.08 (d,  $J$  = 8.7 Hz, 2H), 6.90 (d,  $J$  = 8.7 Hz, 2H), 5.69 (d,  $J$  = 5.7 Hz, 1H), 5.03-4.95 (m, 2H), 4.20 (m, 2H), 3.90-3.65 (m overlapping s, 9H), 3.41 (m, 2H), 3.18-2.78 (m, 5H), 2.44 (m, 1H), 2.00 (m, 1H), 1.61-1.38 (m, 5H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  18.0; MS (ESI) 767 (M+Na).

Example 28

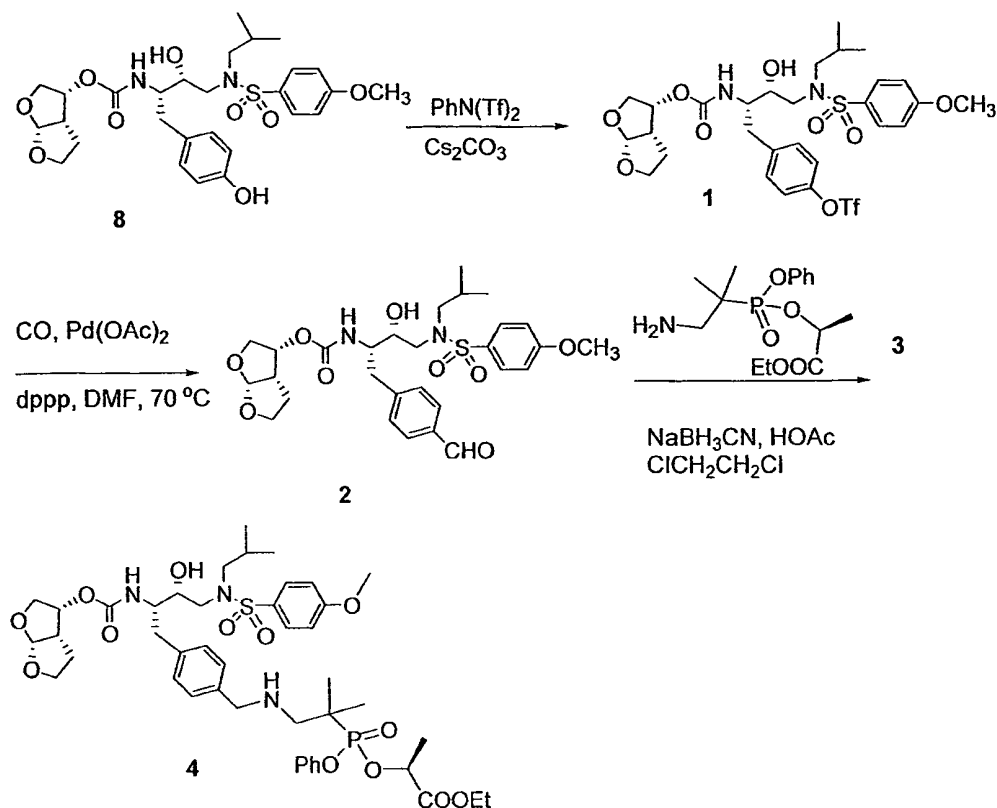
Synthesis of Bislactate 36: A solution of phosphonic acid 11 (100 mg, 0.15 mmol) isopropyl-(S)-lactate (79 mg, 0.66 mmol) was dissolved in pyridine (1 mL) and the solvent was distilled under reduced pressure at 40-60°C. The residue was treated with a solution of Ph<sub>3</sub>P (137 mg, 0.53 mmol) and 2,2'-dipyridyl disulfide (116 mg, 0.53 mmol) in pyridine (1 mL) stirring for 20h at room temperature. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (1% to 5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>). The purified product was suspended in ether and was evaporated under reduced pressure to afford bislactate 36 (42 mg, 32%) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.7 Hz, 2H), 7.01 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 5.66 (d, J = 5.1 Hz, 1H), 5.05 (m, 3H), 4.25 (d, J = 9.9 Hz, 2H), 4.19 (q, 4H), 3.99-3.65 (m overlapping s, 9H), 3.41 (m, 1H), 3.20-2.81 (m, 7H), 1.85-1.60 (m, 3H), 1.58 (m, 6H), 1.26 (m, 12H), 0.93 (d, J = 6.3 Hz, 3H), 0.89 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 21.1; MS (ESI) 923 (M+Na).

Example 29

Triflate derivative 1: A THF-CH<sub>2</sub>Cl<sub>2</sub> solution (30mL-10 mL) of **8** (4 g, 6.9 mmol), cesium carbonate (2.7 g, 8 mmol), and N-phenyltrifluoromethane sulfonimide (2.8 g, 8 mmol) was reacted overnight. The reaction mixture was worked up, and concentrated to dryness to give crude triflate derivative 1.

Aldehyde 2: Crude triflate **1** (4.5 g, 6.9 mmole) was dissolved in DMF (20 mL), and the solution was degassed (high vacuum for 2 min, Ar purge, repeat 3 times). Pd(OAc)<sub>2</sub> (0.12 g, 0.27 mmol), and bis(diphenylphosphino)propane (dppp, 0.22 g, 0.27 mmol) were added and the solution was heated to 70°C. Carbon monoxide was rapidly bubbled through the solution, then under 1 atmosphere of carbon monoxide. To this solution were slowly added TEA (5.4 mL, 38 mmol), and triethylsilane (3 mL, 18 mmol). The resulting solution was stirred overnight at room temperature. The reaction mixture was worked up, and purified on silica gel column chromatograph to afford aldehyde **2** (2.1 g, 51%). (Hostettler, et al. J. Org. Chem., 1999. 64, 178-185).

Lactate prodrug 4: Compound 4 is prepared as described above procedure for 3a-e by the reductive amination between 2 and 3 with  $\text{NaBH}_3\text{CN}$  in 1,2-dichloroethane in the presence of HOAc.



### Example 30

Preparation of compound 3 Diethyl (cyano(dimethyl)methyl) phosphonate 5: A THF solution (30 mL) of NaH (3.4 g of 60% oil dispersion, 85 mmole) was cooled to  $-10^\circ\text{C}$ , followed by the addition of diethyl (cyanomethyl)phosphonate (5g, 28.2 mmol) and iodomethane (17 g, 112 mmol). The resulting solution was stirred at  $-10^\circ\text{C}$  for 2 hr, then  $0^\circ\text{C}$  for 1 hr, was worked up, and purified to give dimethyl derivative 5 (5 g, 86%).

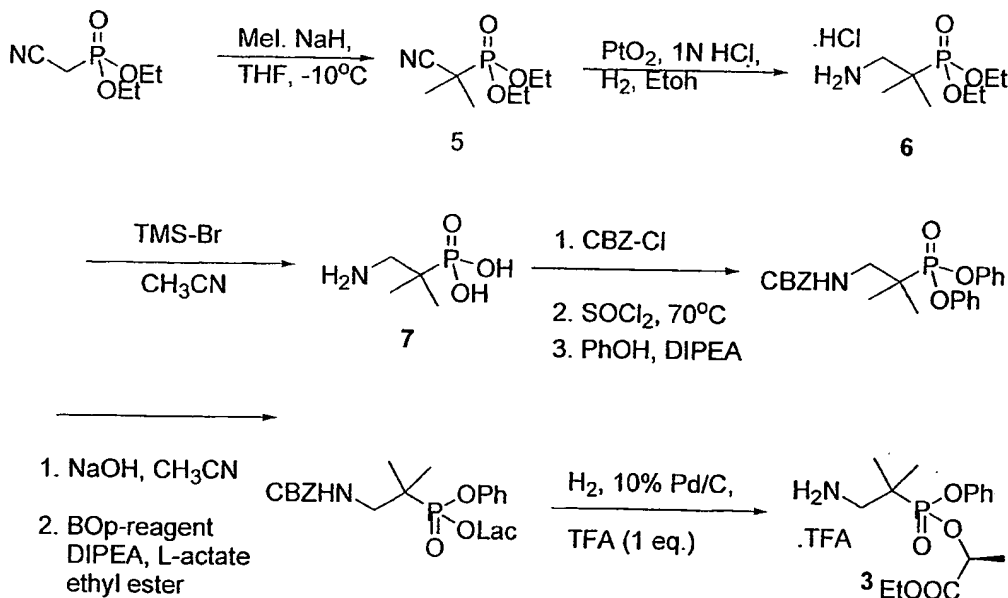
Diethyl (2-amino-1,1-dimethyl-ethyl)phosphonate 6: Compound 5 was reduced to amine derivative 6 by the described procedure (J. Med. Chem. 1999, 42, 5010-5019).

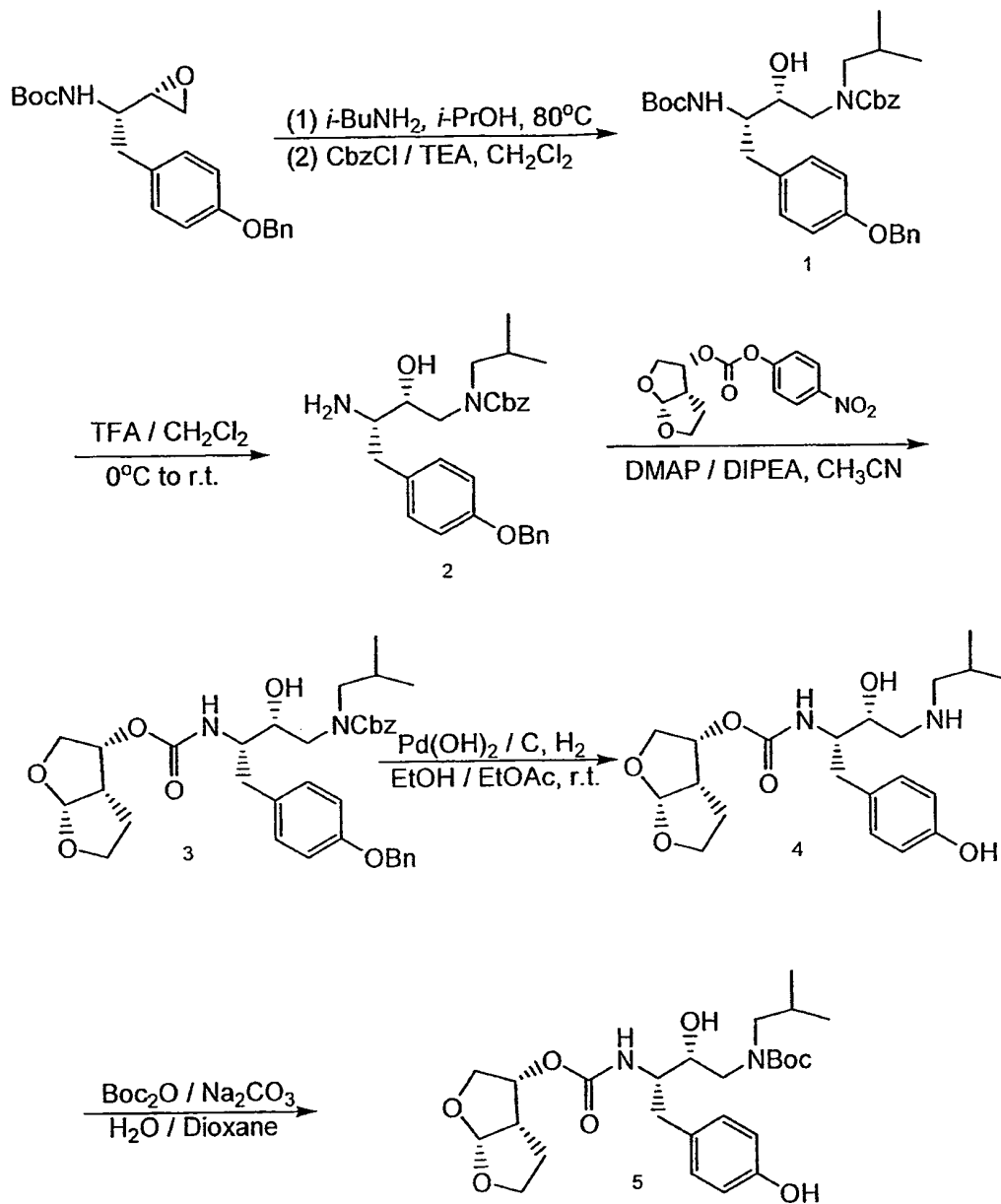
A ethanol (150 mL) and 1N HCl aqueous solution (22 mL) of 5 (2.2 g, 10.7 mmol) was hydrogenated at 1 atmosphere in the presence of  $\text{PtO}_2$  (1.25 g) at room temperature

overnight. The catalyst was filtered through a celite pad. The filtrate was concentrated to dryness, to give crude **6** (2.5g, as HCl salt).

**2-Amino-1,1-dimethyl-ethyl phosphonic acid 7:** A  $\text{CH}_3\text{CN}$  (30 mL) of crude **6** (2.5 g) was cooled to  $0^\circ\text{C}$ , and treated with  $\text{TMSBr}$  (8 g, 52 mmol) for 5 hr. The reaction mixture was stirred with methanol for 1.5 hr at room temperature, concentrated, recharged with methanol, concentrated to dryness to give crude **7** which was used for next reaction without further purification.

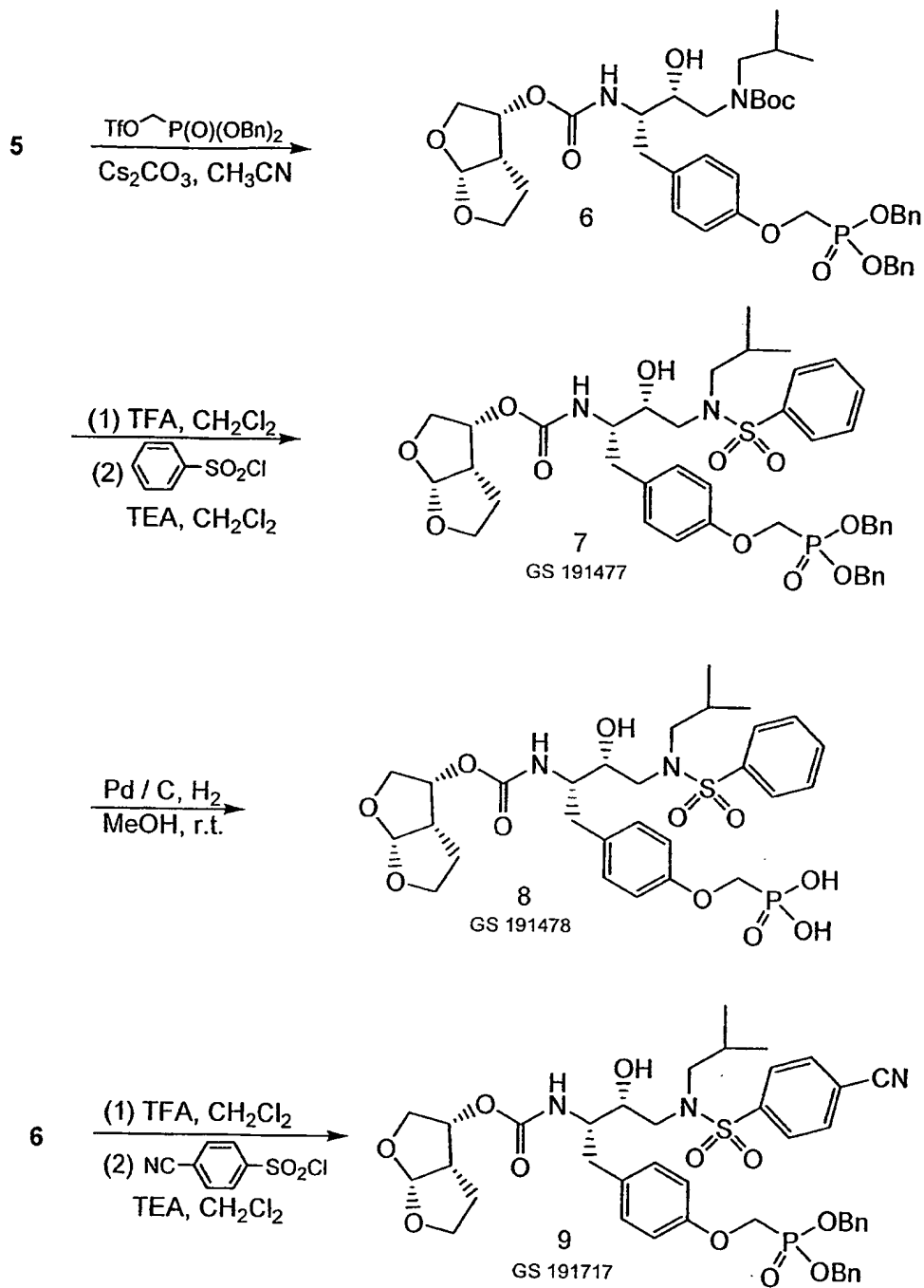
**Lactate phenyl (2-amino-1,1-dimethyl-ethyl)phosphonate 3:** Compound **3** is synthesized according to the procedures described in a previous scheme for the preparation of a lactate phenyl 2-aminoethyl phosphonate. Compound **7** is protected with CBZ, followed by the reaction with thionyl chloride at  $70^\circ\text{C}$ . The CBZ protected dichlorodate is reacted phenol in the presence of DIPEA. Removal of one phenol, follow by coupling with ethyl L-lactate leads N-CBZ-2-amino-1,1-dimethyl-ethyl phosphonated derivative. Hydrogenation of N-CBZ derivative at 1 atmosphere in the presence of 10% Pd/C and 1 equivalent of TFA affords compound **3** as TFA salt.



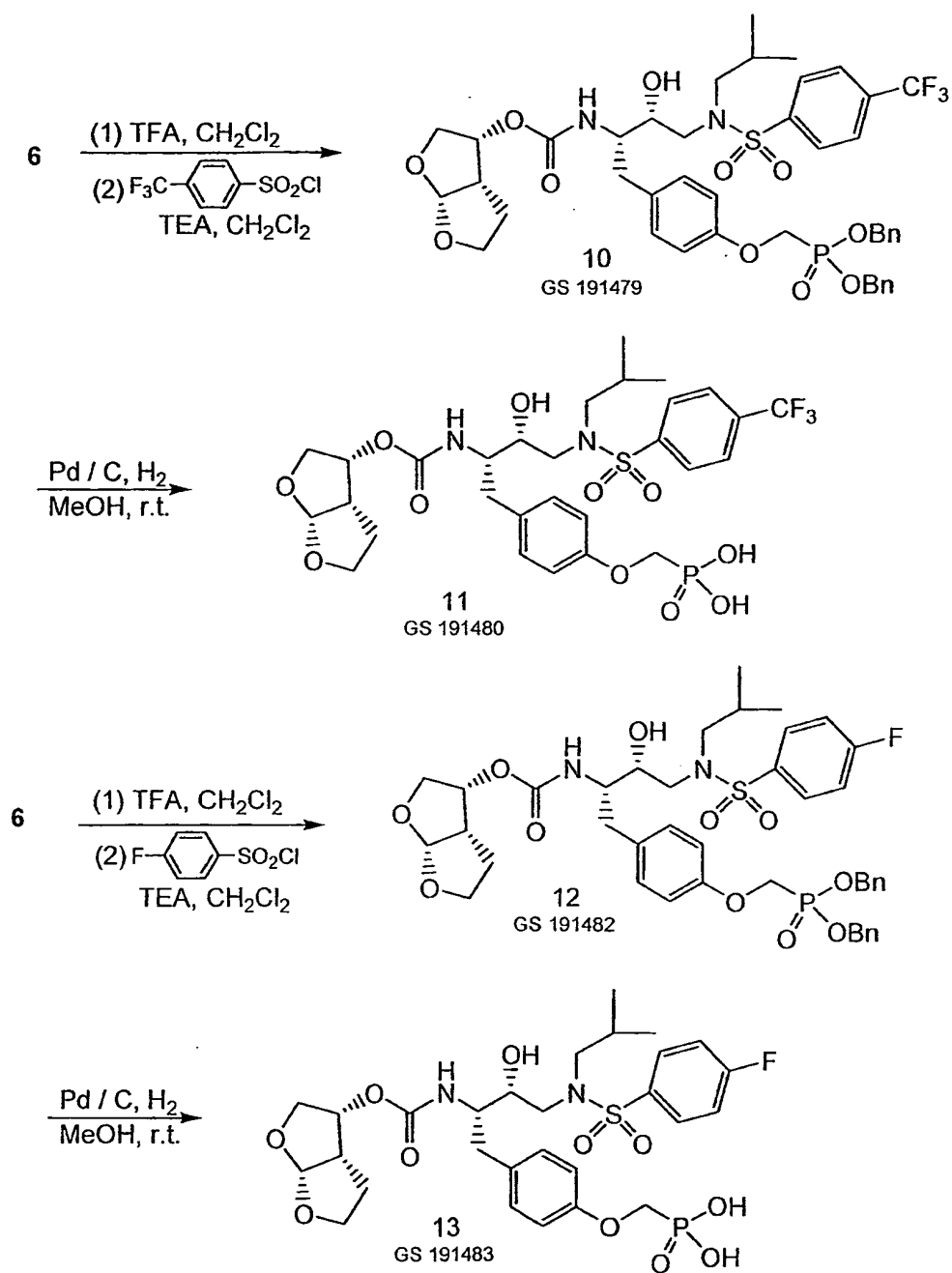
**Example Section M****Scheme 1**



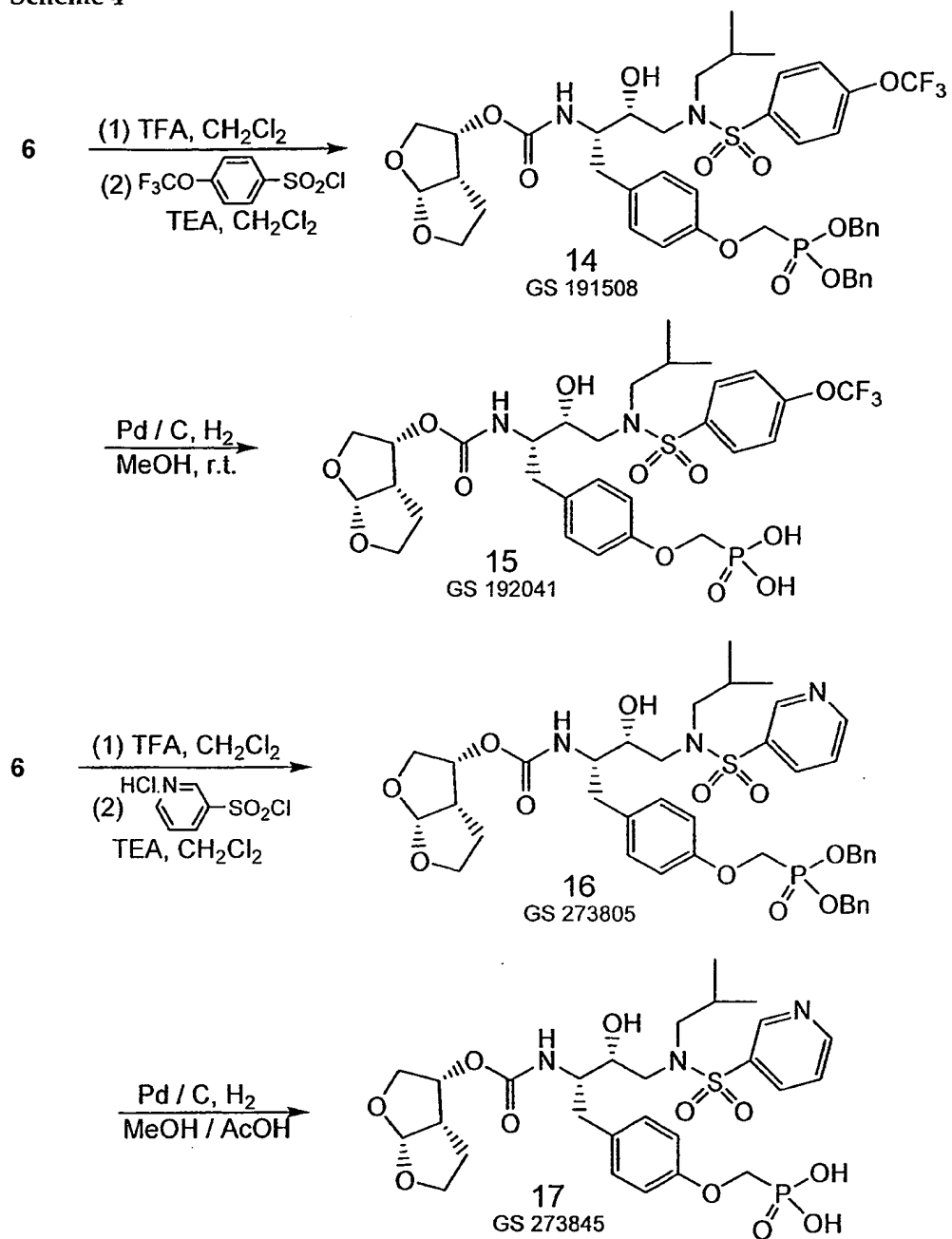
Scheme 2



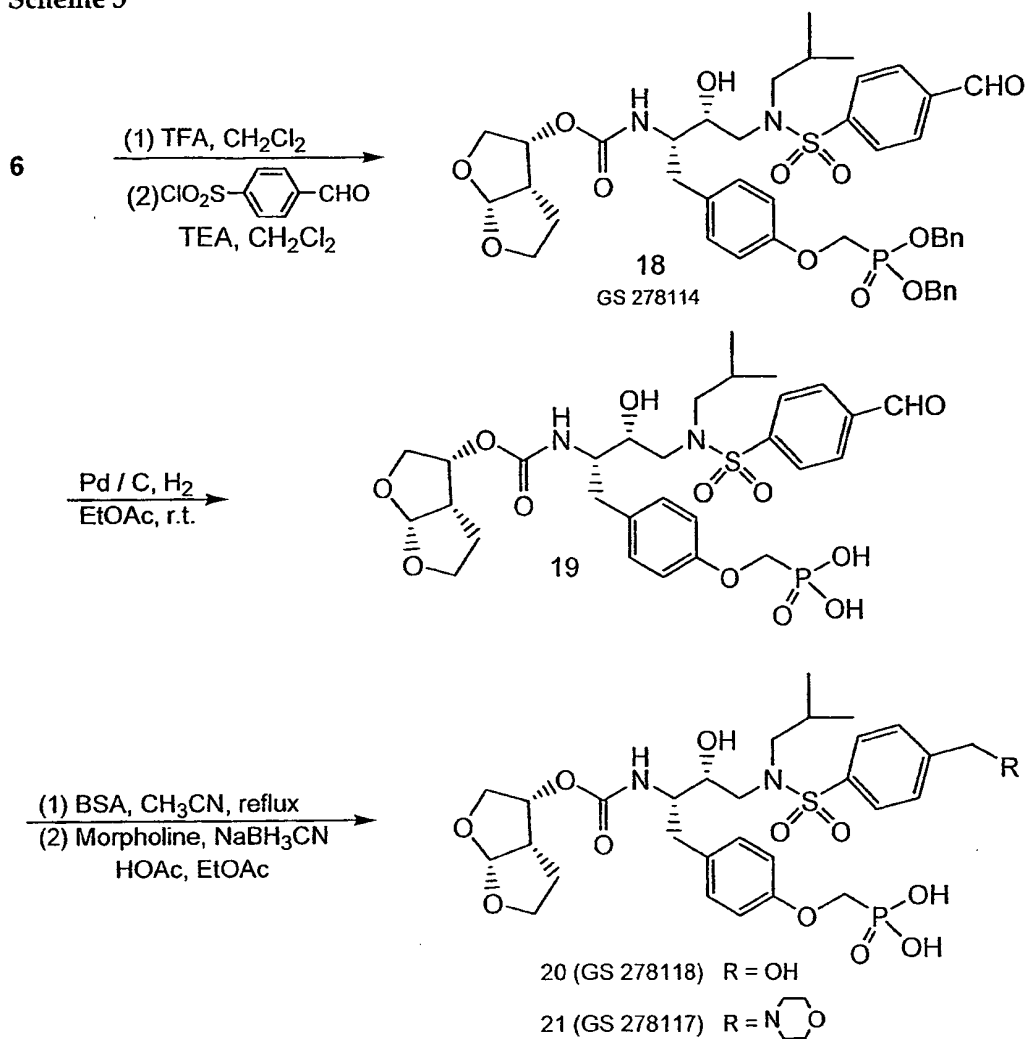
Scheme 3



Scheme 4



Scheme 5

Example 1

**Cbz Amide 1:** To a suspension of epoxide (34 g, 92.03 mmol) in 2-propanol (300 mL) was added isobutylamine (91.5 mL, 920 mmol) and the solution was refluxed for 1 h. The solution was evaporated under reduced pressure and the crude solid was dried under vacuum to give the amine (38.7 g, 95%) which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (300 mL) and cooled to 0°C. Triethylamine (18.3 mL, 131 mmol) was added followed by the addition of benzyl chloroformate (13.7 mL, 96.14 mmol) and the solution was stirred for 30 min at 0°C, warmed to room temperature overnight, and evaporated under reduced pressure. The residue was partitioned between EtOAc and 0.5 M H<sub>3</sub>PO<sub>4</sub>. The organic phase was washed with saturated NaHCO<sub>3</sub>, brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The

crude product was purified by column chromatography on silica gel (1/2-EtOAc/hexane) to give the Cbz amide (45.37 g, 90%) as a white solid.

#### Example 2

- i Amine 2: A solution of Cbz amide 1 (45.37 g, 78.67 mmol) in  $\text{CH}_2\text{Cl}_2$  (160 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (80 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. Volatiles were evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2 x), water (2 x), saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure to give the amine (35.62 g, 95%) as a white solid.

#### Example 3

Carbamate 3: To a solution of amine 2 (20.99 g, 44.03 mmol) in  $\text{CH}_3\text{CN}$  (250 mL) at  $0^\circ\text{C}$  was treated with (3R, 3aR, 6aS)-hexahydrofuro[2, 3-*b*]furan-2-yl 4-nitrophenyl carbonate (13.00 g, 44.03 mmol, prepared according to Ghosh et al. J. Med. Chem. 1996, 39, 3278.), *N,N*-diisopropylethylamine (15.50 mL, 88.06 mmol) and 4-dimethylaminopyridine (1.08 g, 8.81 mmol). The reaction mixture was stirred at  $0^\circ\text{C}$  for 30 min and then warmed to room temperature overnight. The reaction solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and 0.5 N NaOH. The organic phase was washed with 0.5 N NaOH (2 x), 5% citric acid (2 x), saturated  $\text{NaHCO}_3$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the carbamate (23.00 g, 83%) as a white solid.

#### Example 4

Amine 4: To a solution of 3 (23.00 g, 36.35 mmol) in EtOH (200 mL) and EtOAc (50 mL) was added 20%  $\text{Pd}(\text{OH})_2/\text{C}$  (2.30 g). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 3 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the amine (14.00 g, 94%) as a white solid.

Example 5

Phenol 5: To a solution of amine 4 (14.00 g, 34.27 mmol) in H<sub>2</sub>O (80 mL) and 1,4-dioxane (80 mL) at 0°C was added Na<sub>2</sub>CO<sub>3</sub> (5.09 g, 47.98 mmol) and di-*tert*-butyl dicarbonate (8.98 g, 41.13 mmol). The reaction mixture was stirred at 0°C for 2 h and then warmed to room temperature for 30 min. The residue was partitioned between EtOAc and H<sub>2</sub>O. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give the phenol (15.69 g, 90%) as a white solid.

Example 6

Dibenzylphosphonate 6: To a solution of phenol 5 (15.68 g, 30.83 mmol) in CH<sub>3</sub>CN (200 mL) was added Cs<sub>2</sub>CO<sub>3</sub> (15.07 g, 46.24 mmol) and triflate (17.00 g, 40.08 mmol). The reaction mixture was stirred at room temperature for 1 h, the salt was filtered off, and the solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and saturated NaCl. The organic phase was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the dibenzylphosphonate (15.37 g, 73%) as a white solid.

Example 7

Sulfonamide 7: A solution of dibenzylphosphonate 6 (0.21 g, 0.26 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.5 mL) at 0°C was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0°C.

Triethylamine (0.15 mL, 1.04 mmol) was added followed by the treatment of benzenesulfonyl chloride (47 mg, 0.26 mmol). The solution was stirred for 1 h at 0°C and the product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the sulfonamide 7 (0.12 g, 55%, GS 191477) as a white solid: <sup>1</sup>HNMR (CDCl<sub>3</sub>) δ 7.79 (dd, 2H), 7.61-7.56 (m, 3H), 7.38-7.36 (m, 10H), 7.13 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.18 (m, 4H), 5.05 (m, 1H), 4.93 (d, J

= 8.7 Hz, 1H), 4.20 (d,  $J$  = 10.2 Hz, 2H), 4.0-3.67 (m, 7H), 3.15-2.8 (m, 7H), 1.84 (m, 1H), 1.65-1.59 (m, 2H), 0.93 (d,  $J$  = 6.6 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.36.

i Example 8

Phosphonic Acid 8: To a solution of 7 (70 mg, 0.09 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (49 mg, 90% GS 191478) as a white solid:  $^1\text{H}$ NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.83 (dd, 2H), 7.65-7.56 (m, 3H), 7.18 (d,  $J$  = 8.4 Hz, 2H), 6.91 (d,  $J$  = 7.8 Hz, 2H), 5.59 (d,  $J$  = 5.4 Hz, 1H), 4.96 (m, 1H), 4.15 (d,  $J$  = 9.9 Hz, 2H), 3.95-3.68 (m, 6H), 3.44 (dd, 2H), 3.16 (m, 2H), 2.99-2.84 (m, 4H), 2.48 (m, 1H), 2.02 (m, 1H), 1.6 (m, 1H), 1.37 (m, 1H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.87 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.45.

Example 9

Sulfonamide 9: A solution of dibenzylphosphonate 6 (0.24 g, 0.31 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ .

Triethylamine (0.17 mL, 1.20 mmol) was added followed by the treatment of 4-cyanobenzenesulfonyl chloride (61.4 mg, 0.30 mmol). The solution was stirred for 1 h at  $0^\circ\text{C}$  and the product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the sulfonamide 9 (0.20 g, 77%, GS 191717) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.90 (d,  $J$  = 8.4 Hz, 2H), 7.83 (d,  $J$  = 7.8 Hz, 2H), 7.36 (m, 10H), 7.11 (d,  $J$  = 8.4 Hz, 2H), 6.82 (d,  $J$  = 8.7 Hz, 2H), 5.65 (d,  $J$  = 5.4 Hz, 1H), 5.2-4.9 (m, 5H), 4.8 (d, 1H), 4.2 (d,  $J$  = 9.9 Hz, 2H), 3.99 (m 1H), 3.94 (m, 3H), 3.7 (m, 2H), 3.48 (broad, s, 1H), 3.18-

2.78 (m, 7H), 1.87 (m, 1H), 1.66-1.47 (m, 2H), 0.91 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3..

#### Example 10

- 5 Sulfonamide 10: A solution of dibenzylphosphonate 6 (0.23 g, 0.29 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.16 mL, 1.17 mmol) was added followed by the treatment of 4-trifluoromethyl benzenesulfonyl chloride (72 mg, 0.29 mmol). The solution was stirred for 1 h at  $0^\circ\text{C}$  and the product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the sulfonamide (0.13 g, 50%, GS 191479) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.92 (d, J = 8.1 Hz, 2H), 7.81 (d, J = 8.1 Hz, 2H), 7.36 (m, 10H), 7.12 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 5.65 (d, J = 5.1 Hz, 1H), 5.20-4.89 (m, 6H), 4.20 (d, J = 9.9 Hz, 2H), 3.95 (m, 1H), 3.86 (m, 3H), 3.71 (m, 2H), 3.19-2.78 (m, 7H), 1.86 (m, 1H), 1.65 (m, 2H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

#### Example 11

Phosphonic Acid 11: To a solution of 10 (70 mg, 0.079 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (50 mg, 90%, GS 191480) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.03 (dd, 2H), 7.90 (dd, 2H), 7.17 (d, J = 8.1 Hz, 2H), 6.91 (d, J = 7.8 Hz, 2H), 5.59 (d, J = 5.7 Hz, 1H), 4.94 (m, 1H), 4.15 (d, J = 10.2 Hz, 2H), 3.94-3.72 (m, 6H), 3.48 (m, 1H), 3.2-3.1 (m, 3H), 3.0-2.9 (m, 2H), 2.47 (m, 1H), 2.06 (m, 1H), 1.56 (m, 1H), 1.37 (m, 1H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.5.



### Example 12

Sulfonamide 12: A solution of dibenzylphosphonate 6 (0.23 g, 0.29 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ .

Triethylamine (0.16 mL, 1.17 mmol) was added followed by the treatment of 4-fluorobenzenesulfonyl chloride (57 mg, 0.29 mmol). The solution was stirred for 1 h at  $0^\circ\text{C}$  and the product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated  $\text{NaCl}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the sulfonamide (0.13 g, 55%, GS 191482) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.81 (m, 2H), 7.38 (m, 10H), 7.24 (m, 2H), 7.12 (d,  $J = 8.1$  Hz, 2H), 6.82 (d,  $J = 8.4$  Hz, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.17 (m, 4H), 5.0 (m, 1H), 4.90 (d, 1H), 4.20 (d,  $J = 9.9$  Hz, 2H), 3.97 (m, 1H), 3.86 (m, 3H), 3.73 (m, 2H), 3.6 (broad, s, 1H), 3.13 (m, 1H), 3.03-2.79 (m, 6H), 1.86 (m, 1H), 1.66-1.58 (m, 2H), 0.92 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

### Example 13

Phosphonic Acid 13: To a solution of 12 (70 mg, 0.083 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (49 mg, 90%, GS 191483) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.89 (m, 2H), 7.32 (m, 2H), 7.18 (d,  $J = 8.4$  Hz, 2H), 6.9 (d,  $J = 8.1$  Hz, 2H), 5.59 (d,  $J = 5.1$  Hz, 1H), 4.94 (m, 1H), 4.16 (d,  $J = 9.9$  Hz, 2H), 3.94 (m, 1H), 3.85-3.7 (m, 5H), 3.43 (dd, 1H), 3.15-2.87 (m, 5H), 2.48 (m, 1H), 2.03 (m, 1H), 1.59-1.36 (m, 2H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.87 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.5.

Example 14

Sulfonamide 14: A solution of dibenzylphosphonate 6 (0.21 g, 0.26 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ .

Triethylamine (0.15 mL, 1.04 mmol) was added followed by the treatment of 4-trifluoromethoxybenzenesulfonyl chloride (69 mg, 0.26 mmol). The solution was stirred for 1 h at  $0^\circ\text{C}$  and the product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the sulfonamide (0.17 g, 70%, GS 191508) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.84 (d,  $J$  = 9 Hz, 2H), 7.36 (m, 12H), 7.12 (d,  $J$  = 8.7 Hz, 2H), 6.81 (d,  $J$  = 8.7 Hz, 2H), 5.65 (d,  $J$  = 5.4 Hz, 1H), 5.16 (m, 4H), 5.03 (m, 1H), 4.89 (d, 1H), 4.2 (d,  $J$  = 9.9 Hz, 2H), 3.97 (m, 1H), 3.85 (m, 3H), 3.7 (m, 2H), 3.59 (broad, s, 1H), 3.18 (m, 1H), 3.1-3.0 (m, 3H), 2.96-2.78 (m, 3H), 1.86 (m, 1H), 1.66-1.5 (m, 2H), 0.93 (d,  $J$  = 6.6 Hz, 3H), 0.88 (d,  $J$  = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

Example 15

Phosphonic Acid 15: To a solution of 14 (70 mg, 0.083 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (50 mg, 90%, GS 192041) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.95 (dd, 2H), 7.49 (dd, 2H), 7.17 (dd, 2H), 6.92 (dd, 2H), 5.58 (d,  $J$  = 5.4 Hz, 1H), 4.89 (m, 1H), 4.17 (d,  $J$  = 9 Hz, 2H), 3.9 (m, 1H), 3.82-3.7 (m, 5H), 3.44 (m, 1H), 3.19-2.9 (m, 5H), 2.48 (m, 1H), 2.0 (m, 1H), 1.6 (m, 1H), 1.35 (m, 1H), 0.93 (d,  $J$  = 6.0 Hz, 3H), 0.88 (d,  $J$  = 6.0 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.4.

Example 16

Sulfonamide 16: A solution of dibenzylphosphonate 6 (0.59 g, 0.76 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.0 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (1.0 mL). The solution was stirred for 30

min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0°C.

Triethylamine (0.53 mL, 3.80 mmol) was added followed by the treatment of hydrogen chloride salt of 3-pyridinylsulfonyl chloride (0.17 g, 0.80 mmol, prepared according to Karaman, R. et al. J. Am. Chem. Soc. 1992, 114, 4889). The solution was stirred for 30 min at 0°C and warmed to room temperature for 30 min. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and saturated NaHCO<sub>3</sub>. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (4% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the sulfonamide (0.50 g, 80%, GS 273805) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 9.0 (d, J = 1.5 Hz, 1H), 8.8 (dd, 1H), 8.05 (d, J = 8.7 Hz, 1H), 7.48 (m, 1H), 7.36 (m, 10H), 7.12 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 9.0 Hz, 2H), 5.65 (d, J = 5.1 Hz, 1H), 5.18 (m, 4H), 5.06 (m, 1H), 4.93 (d, 1H), 4.21 (d, J = 8.4 Hz, 2H), 3.97 (m, 1H), 3.86 (m, 3H), 3.74 (m, 2H), 3.2 (m, 1H), 3.1-2.83 (m, 5H), 2.76 (m, 1H), 1.88 (m, 1H), 1.62 (m, 2H), 0.92 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.3.

#### Example 17

Phosphonic Acid 17: To a solution of 16 (40 mg, 0.049 mmol) in MeOH (3 mL) and AcOH (1 mL) was added 10% Pd/C (10 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (28 mg, 90%, GS 273845) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.98 (s, 1H), 8.77 (broad, s, 1H), 8.25 (dd, 1H), 7.6 (m, 1H), 7.15 (m, 2H), 6.90 (m, 2H), 5.6 (d, J = 5.4 Hz, 1H), 4.98 (m, 1H), 4.15 (d, 2H), 3.97-3.7 (m, 6H), 3.45-2.89 (m, 6H), 2.50 (m, 1H), 2.0 (m, 1H), 1.6-1.35 (m, 2H), 0.9 (m, 6H).

#### Example 18

Sulfonamide 18: A solution of dibenzylphosphonate 6 (0.15 g, 0.19 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.60 mL) at 0°C was treated with trifluoroacetic acid (0.30 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was

co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to  $0^\circ\text{C}$ .

Triethylamine (0.11 mL, 0.76 mmol) was added followed by the treatment of 4-formylbenzenesulfonyl chloride (43 mg, 0.21 mmol). The solution was stirred for 30 min at  $0^\circ\text{C}$  and warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated  $\text{NaCl}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the sulfonamide (0.13 g, 80%, GS 278114) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.1 (s, 1H), 8.04 (d,  $J = 8.1$  Hz, 2H), 7.94 (d,  $J = 8.1$  Hz, 2H), 7.35 (m, 10H), 7.13 (m,  $J = 8.1$  Hz, 2H), 6.82 (d,  $J = 8.1$  Hz, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.17 (m, 4H), 5.06 (m, 1H), 4.93 (m, 1H), 4.2 (d,  $J = 9.9$  Hz, 2H), 3.94 (m, 1H), 3.85 (m, 3H), 3.7 (m, 2H), 3.18-2.87 (m, 5H), 2.78 (m, 1H), 1.86 (m, 1H), 1.67-1.58 (m, 2H), 0.93 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

#### Example 19

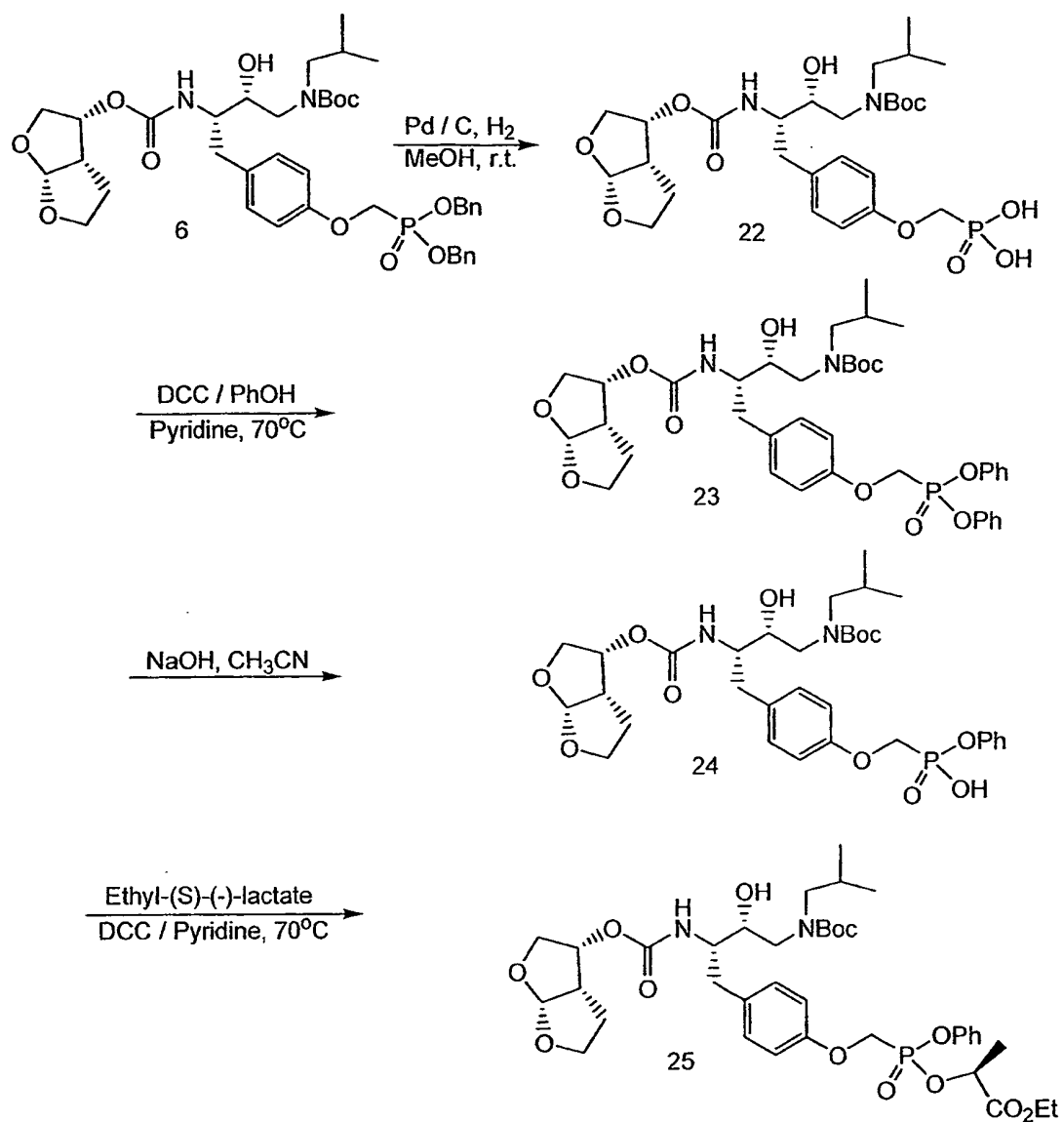
Phosphonic Acid 19: To a solution of 18 (0.12 g, 0.15 mmol) in EtOAc (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 6 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (93 mg, 95%) as a white solid.

#### Example 20

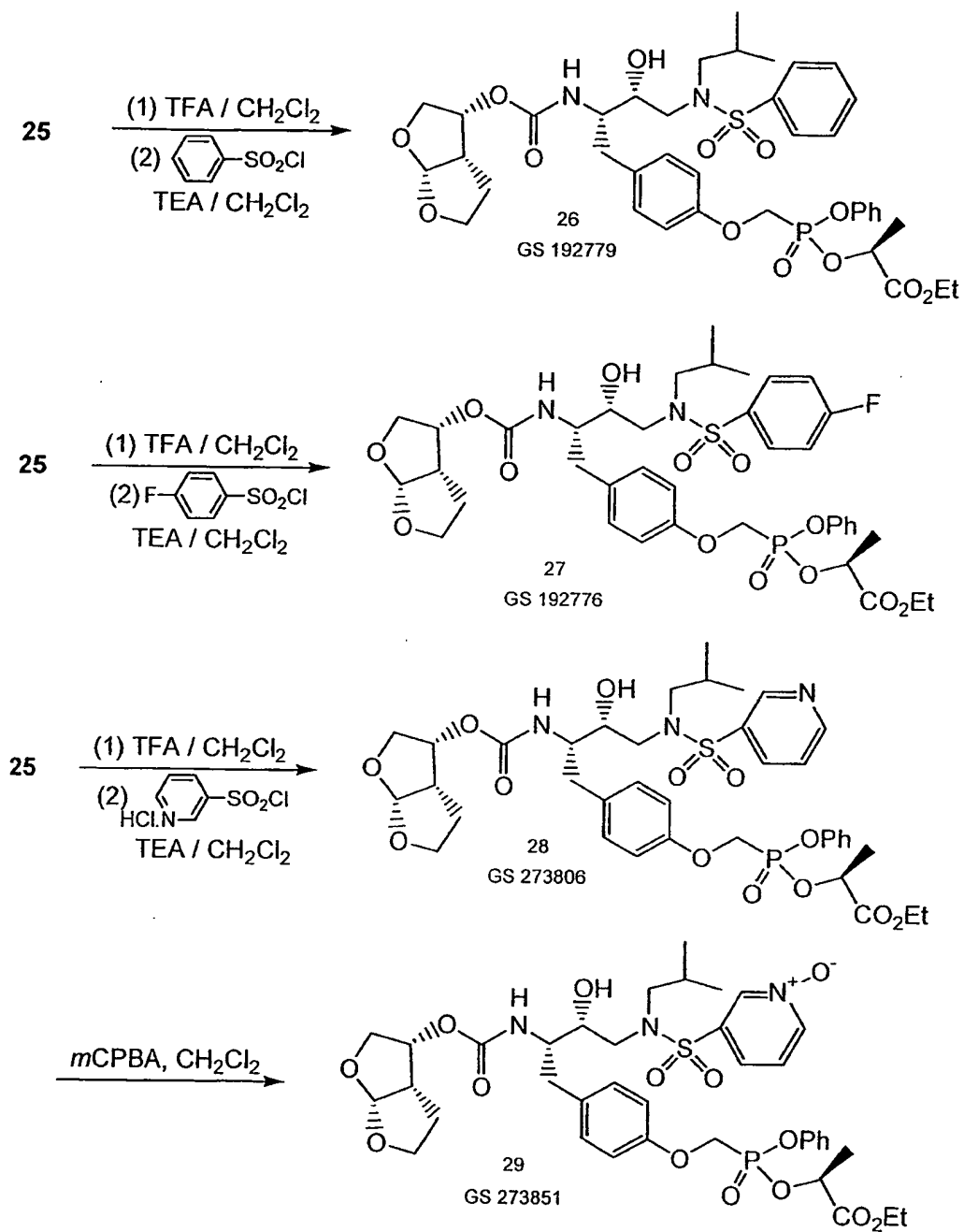
Phosphonic Acids 20 and 21: Compound 19 (93 mg, 0.14 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (2 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.28 g, 1.4 mmol) was added. The reaction mixture was heated to reflux for 1 h, cooled to room temperature and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a semi-solid which was dissolved in EtOAc (2 mL). Morpholine (60  $\mu\text{L}$ , 0.9 mmol), AcOH (32  $\mu\text{L}$ , 0.56 mmol), and  $\text{NaBH}_3\text{CN}$  (17 mg, 0.28 mmol) were added and the reaction mixture was stirred at room temperature overnight. The reaction was quenched with  $\text{H}_2\text{O}$ , stirred for 2 h, filtered, and concentrated. The crude product was purified by HPLC to give the phosphonic acid 20 (10 mg, GS 278118) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.80 (d,  $J =$

7.8 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 7.17 (d, J = 7.8 Hz, 2H), 6.91 (d, J = 7.5 Hz, 2H), 5.59 (d, J = 5.1 Hz, 1H), 5.06 (m, 1H), 4.7 (s, 2H), 4.15 (d, J = 10.2 Hz, 2H), 3.92 (m, 1H), 3.82-3.7 (m, 5H), 3.43 (dd, 1H), 3.11-2.89 (m, 6H), 2.50 (m, 1H), 2.0 (m, 1H), 1.6-1.35 (m, 2H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.3. Phosphonic acid 21 (15 mg, GS 278117) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.8-7.7 (m, 4H), 7.20 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 5.62 (d, J = 5.1 Hz, 1H), 5.00 (m, 1H), 4.42 (s, 2H), 4.20 (dd, 2H), 3.98-3.68 (m, 9H), 3.3-2.92 (m, 11H), 2.6 (m, 1H), 2.0 (m, 1H), 1.6 (m, 2H), 0.92 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  16.2.

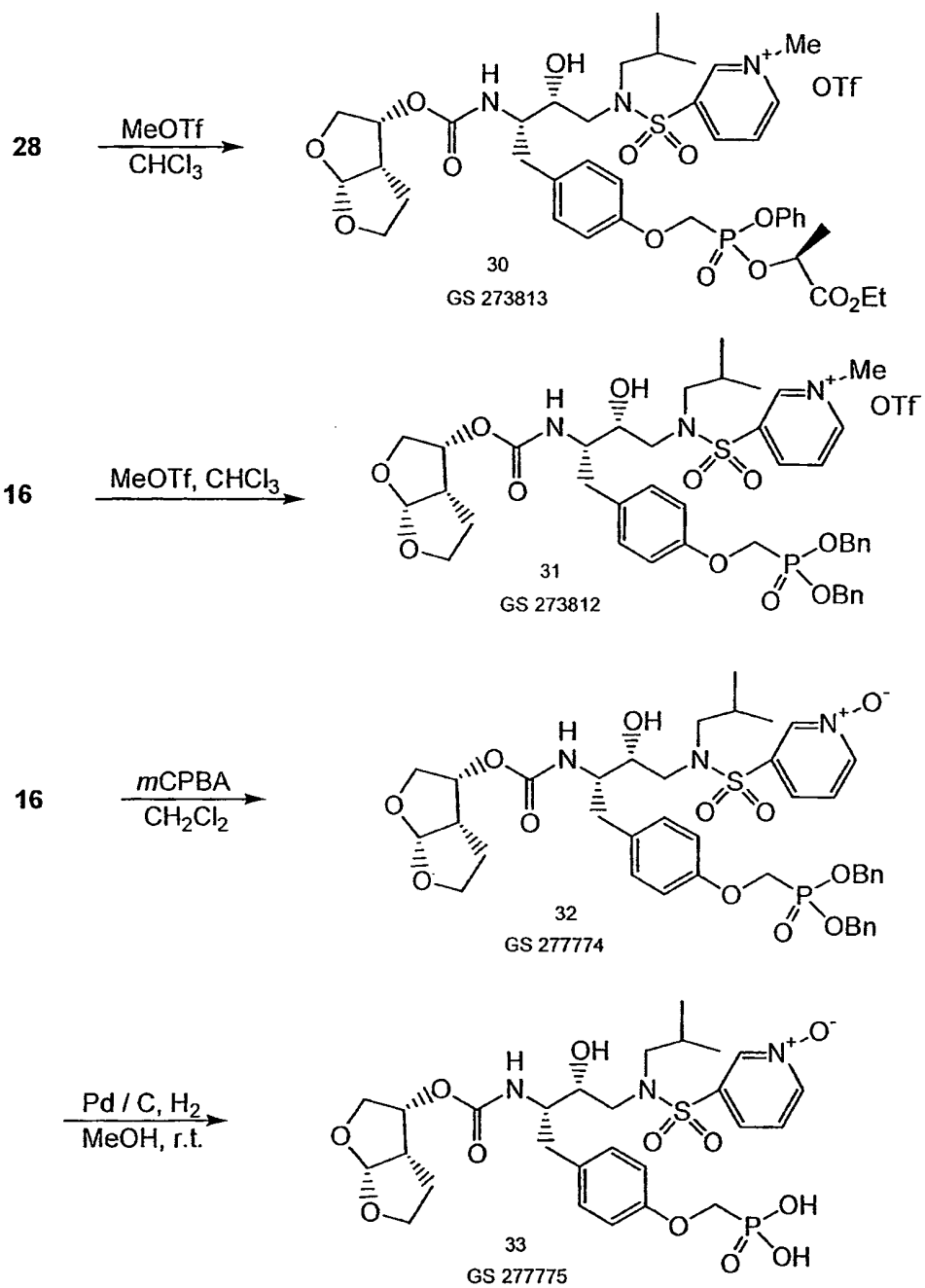
Scheme 6



Scheme 7

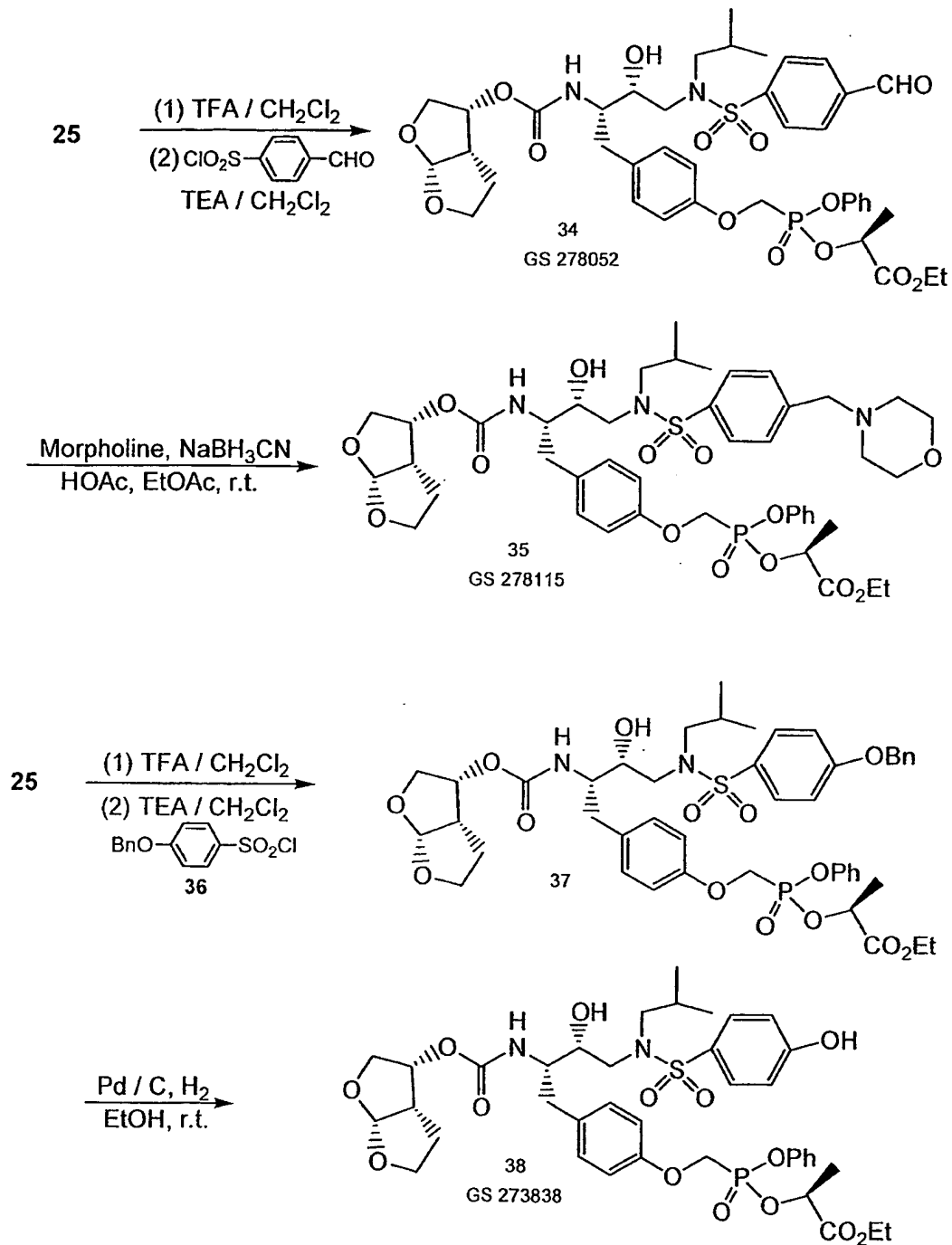


Scheme 8

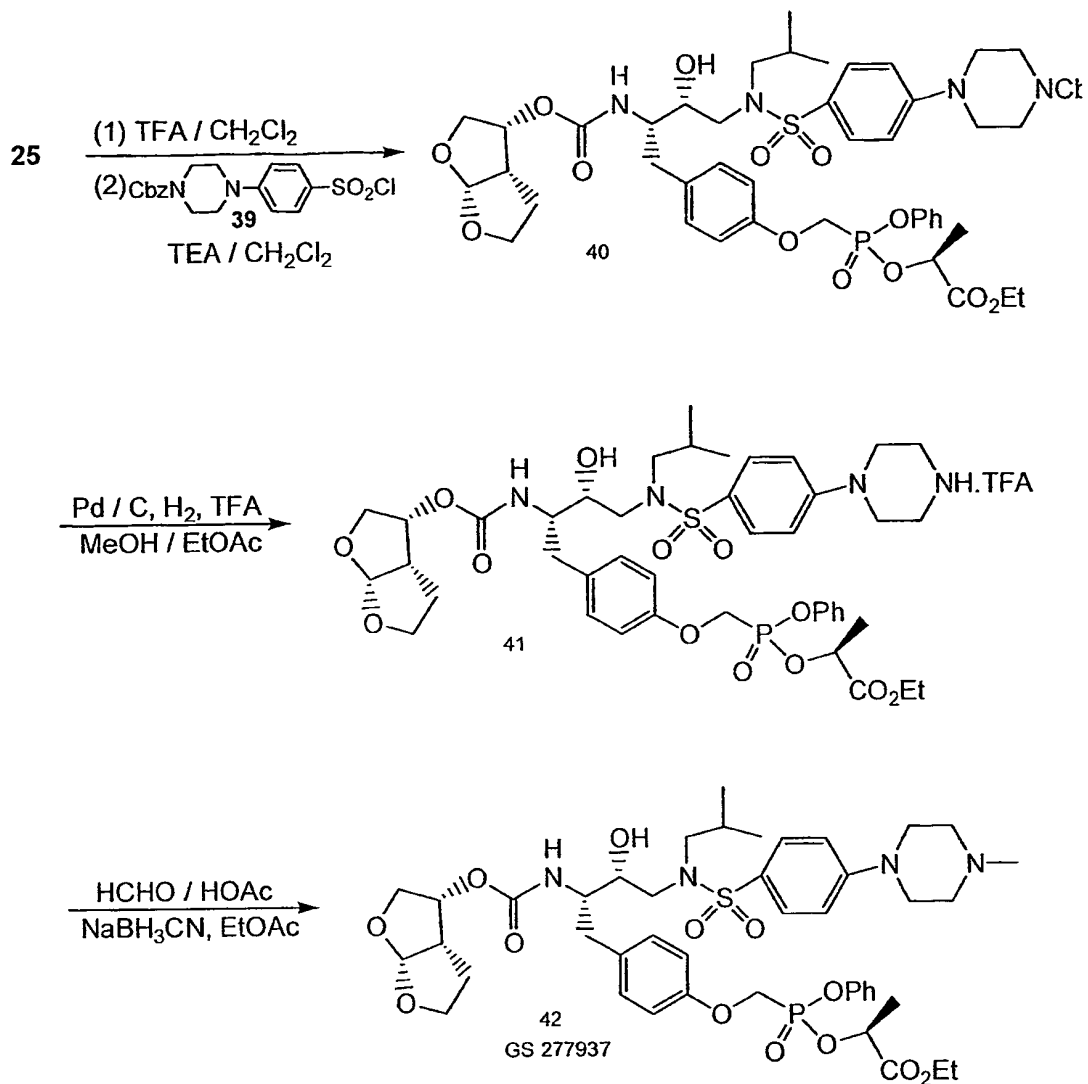




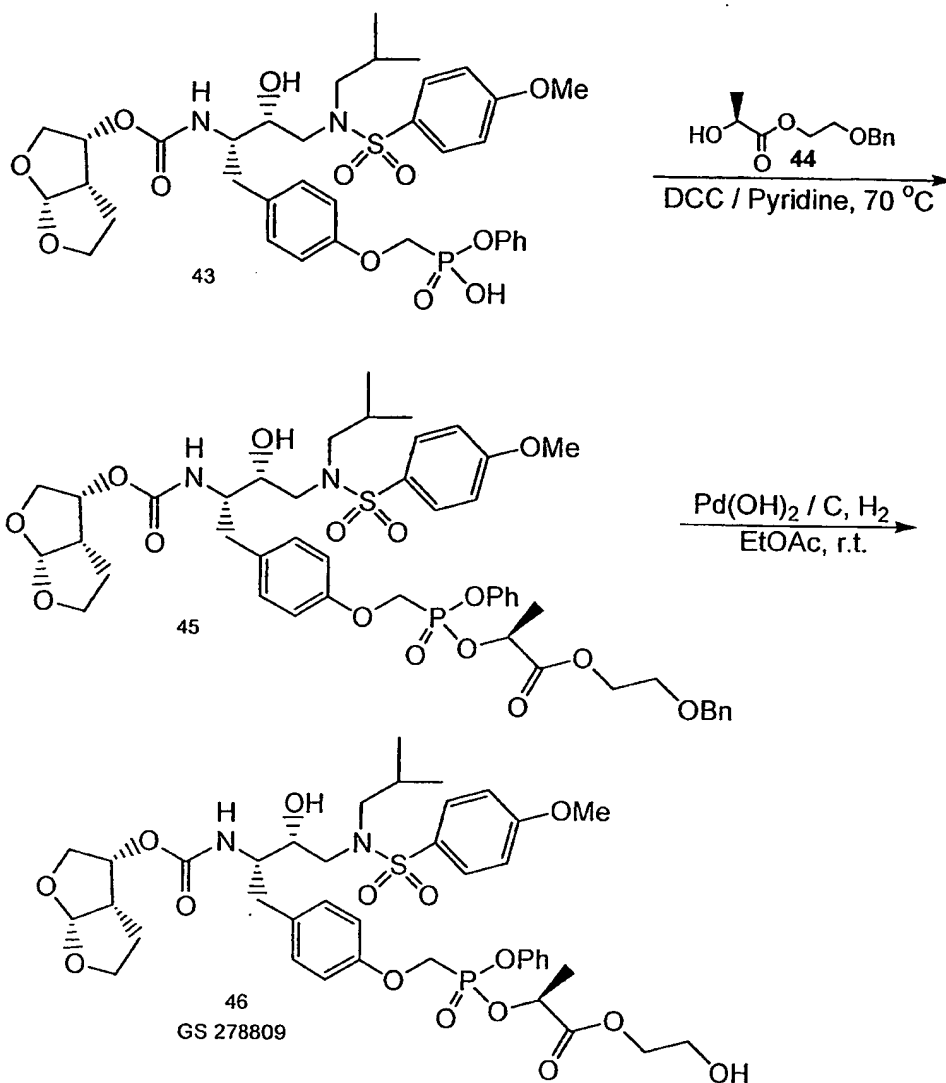
Scheme 9



## Scheme 10



## Scheme 11

Example 21

Phosphonic Acid 22: To a solution of dibenzylphosphonate 6 (5.00 g, 6.39 mmol) in EtOH (100 mL) was added 10% Pd/C (1.4 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (3.66 g, 95%) as a white solid.

Example 22

Diphenylphosphonate 23: A solution of 22 (3.65 g, 6.06 mmol) and phenol (5.70 g, 60.6 mmol) in pyridine (30 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (5.00 g, 24.24 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. EtOAc was added and the side product 1,3-dicyclohexyl urea was filtered off.

- 5 The filtrate was concentrated and dissolved in CH<sub>3</sub>CN (20 mL) at 0°C. The mixture was treated with DOWEX 50W x 8-400 ion-exchange resin and stirred for 30 min at 0°C. The resin was filtered off and the filtrate was concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the diphenylphosphonate (2.74 g, 60%) as a white solid.

#### Example 23

Monophosphonic Acid 24: To a solution of 23 (2.74 g, 3.63 mmol) in CH<sub>3</sub>CN (40 mL) at 0°C was added 1 N NaOH (9.07 mL, 9.07 mmol). The reaction mixture was stirred at 0°C for 1 h. DOWEX 50W x 8-400 ion-exchange resin was added and the reaction mixture was stirred for 30 min at 0°C. The resin was filtered off and the filtrate was concentrated and co-evaporated with toluene. The crude product was triturated with EtOAc/hexane (1/2) to give the monophosphonic acid (2.34 g, 95%) as a white solid.

#### Example 24

Monophospholactate 25: A solution of 24 (2.00 g, 2.95 mmol) and ethyl-(S)-(-)-lactate (1.34 mL, 11.80 mmol) in pyridine (20 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (2.43 g, 11.80 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H<sub>2</sub>O, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (1.38 g, 60%) as a white solid.

#### Example 25

Monophospholactate 26: A solution of 25 (0.37 g, 0.48 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.80 mL) at 0°C was treated with trifluoroacetic acid (0.40 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was

diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ .

Triethylamine (0.27 mL, 1.92 mmol) was added followed by the treatment of

benzenesulfonyl chloride (84 mg, 0.48 mmol). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.2 N HCl. The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.33 g, 85%, GS 192779, 1:1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.78 (dd, 2H), 7.59 (m, 3H), 7.38-7.18 (m, 7H), 6.93 (dd, 2H), 5.66 (m, 1H), 5.18-4.93 (m, 3H), 4.56-4.4 (m, 2H), 4.2 (m, 2H), 4.1-3.7 (m, 6H), 3.17 (m, 1H), 3.02-2.8 (m, 6H), 1.84 (m, 1H), 1.82-1.5 (m, 5H), 1.27 (m, 3H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4, 15.3.

#### Example 26

Monophospholactate 27: A solution of 25 (0.50 g, 0.64 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.0 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (4 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.36 mL, 2.56 mmol) was added followed by the treatment of 4-fluorobenzenesulfonyl chloride (0.13 g, 0.64 mmol). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.2 N HCl. The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.44 g, 81%, GS 192776, 3/2 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.80 (m, 2H), 7.38-7.15 (m, 9H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-4.9 (m, 3H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.1-3.7 (m, 6H), 3.6 (broad, s, 1H), 3.17 (m, 1H), 3.02-2.75 (m, 6H), 1.85 (m, 1H), 1.7-1.5 (m, 5H), 1.26 (m, 3H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.2.

Example 27

Monophospholactate 28: A solution of 25 (0.50 g, 0.64 mmol) in  $\text{CH}_2\text{Cl}_2$  (1.0 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.45 mL, 3.20 mmol) was added followed by the treatment of hydrogen chloride salt of 3-pyridinylsulfonyl chloride (0.14 g, 0.65 mmol). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.41 g, 79%, GS 273806, 1:1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.0 (s, 1H), 8.83 (dd, 1H), 8.06 (d,  $J = 7.8$  Hz, 1H), 7.5 (m, 1H), 7.38-7.15 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.18-4.95 (m, 3H), 4.6-4.41 (m, 2H), 4.2 (m, 2H), 4.0 (m, 1H), 3.95-3.76 (m, 6H), 3.23-2.8 (m, 7H), 1.88 (m, 1H), 1.7-1.5 (m, 5H), 1.26 (m, 3H), 0.93 (d,  $J = 6.6$  Hz, 3H), 0.83 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.3.

Example 28

Monophospholactate 29: A solution of compound 28 (0.82 g, 1.00 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) at  $0^\circ\text{C}$  was treated with *m*CPBA (1.25 eq). The solution was stirred for 1 h at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 6 h. The reaction mixture was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.59 g, 70%, GS 273851, 1:1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.63 (dd, 1H), 8.3 (dd, 1H), 7.57 (m, 1H), 7.44 (m, 1H), 7.38-7.13 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-5.05 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.0-3.73 (m, 6H), 3.2 (m, 2H), 3.0 (m, 4H), 2.77 (m, 1H), 1.92 (m, 1H), 1.7-1.49 (m, 5H), 1.26 (m, 3H), 0.91 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.3.

Example 29

Monophospholactate 30: A solution of compound 28 (71 mg, 0.087 mmol) in  $\text{CHCl}_3$  (1 mL) was treated with MeOTf (18 mg, 0.11 mmol). The solution was stirred at room temperature for 1 h. The reaction mixture was concentrated and co-evaporated with toluene (2 x),  $\text{CHCl}_3$  (2 x) and dried under vacuum to give the monophospholactate (81 mg, 95%, GS 273813, 1:1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.0 (dd, 1H), 8.76 (m, 2H), 8.1 (m, 1H), 7.35-7.1 (m, 7H), 6.89 (m, 2H), 5.64 (m, 1H), 5.25-5.0 (m, 3H), 4.6-4.41 (m, 5H), 4.2 (m, 2H), 3.92-3.72 (m, 6H), 3.28 (m, 2H), 3.04-2.85 (m, 3H), 2.62 (m, 1H), 1.97 (m, 1H), 1.62-1.5 (m, 5H), 1.25 (m, 3H), 0.97 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4, 15.4.

Example 30

Dibenzylphosphonate 31: A solution of compound 16 (0.15 g, 0.18 mmol) in  $\text{CHCl}_3$  (2 mL) was treated with MeOTf (37 mg, 0.23 mmol). The solution was stirred at room temperature for 2 h. The reaction mixture was concentrated and co-evaporated with toluene (2 x),  $\text{CHCl}_3$  (2 x) and dried under vacuum to give the dibenzylphosphonate (0.17 g, 95%, GS 273812) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.0 (dd, 1H), 8.73 (m, 2H), 8.09 (m, 1H), 7.35 (m, 10H), 7.09 (d,  $J = 8.4$  Hz, 2H), 6.79 (d,  $J = 8.1$  Hz, 2H), 5.61 (d,  $J = 4.2$  Hz, 1H), 5.2-4.96 (m, 6H), 4.54 (s, 3H), 4.2 (dd, 2H), 3.92-3.69 (m, 6H), 3.3 (m, 2H), 3.04-2.6 (m, 5H), 1.97 (m, 1H), 1.6 (m, 2H), 0.98 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.4.

Example 31

Dibenzylphosphonate 32: A solution of compound 16 (0.15 g, 0.18 mmol) in  $\text{CH}_2\text{Cl}_2$  (3 mL) at  $0^\circ\text{C}$  was treated with *m*CPBA (1.25 eq). The solution was stirred for 1 h at  $0^\circ\text{C}$  and then warmed to room temperature overnight. The reaction mixture was partitioned between 10% 2-propanol/ $\text{CH}_2\text{Cl}_2$  and saturated  $\text{NaHCO}_3$ . The organic phase was washed with saturated  $\text{NaCl}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the dibenzylphosphonate (0.11 g, 70%, GS 277774) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.64 (m, 1H), 8.27 (d,  $J = 6.9$  Hz, 1H), 7.57 (d,  $J = 8.4$  Hz, 1H), 7.36 (m, 11H), 7.10 (d,  $J = 8.4$  Hz, 2H), 6.81 (d,  $J = 8.7$  Hz, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.22-5.02 (m, 6H), 4.21 (dd, 2H), 3.99-3.65 (m, 6H), 3.2 (m, 2H), 3.03-2.73 (m, 5H), 1.90 (m, 1H), 1.66-1.56 (m, 2H), 0.91 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.3.

Example 32

Phosphonic Acid 33: To a solution of dibenzylphosphonate 32 (0.1 g, 0.12 mmol) in MeOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 1 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and purified by HPLC to give the phosphonic acid (17 mg, GS 277775) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.68 (s, 1H), 8.47 (d, J = 6.0 Hz, 1H), 7.92 (d, J = 7.8 Hz, 1H), 7.68 (m, 1H), 7.14 (m, 2H), 6.90 (d, J = 7.8 Hz, 2H), 5.58 (d, J = 5.4 Hz, 1H), 5.00 (m, 1H), 4.08 (d, J = 9.9 Hz, 2H), 3.93-3.69 (m, 6H), 3.4-2.9 (m, 7H), 2.5 (m, 1H), 2.04 (m, 1H), 1.6-1.35 (m, 2H), 0.92 (m, 6H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 15.8.

Example 33

Monophospholactate 34: A solution of 25 (2.50 g, 3.21 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5.0 mL) at 0°C was treated with trifluoroacetic acid (2.5 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and cooled to 0°C. Triethylamine (1.79 mL, 12.84 mmol) was added followed by the treatment of 4-formylbenzenesulfonyl chloride (0.72 g, 3.53 mmol) and the solution was stirred at 0°C for 1 h. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and 5% HCl. The organic phase was washed with H<sub>2</sub>O, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (2.11 g, 77%, GS 278052, 1:1 diastereomeric mixture) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 10.12 (s, 1H), 8.05 (d, J = 8.7 Hz, 2H), 7.95 (d, J = 7.5 Hz, 2H), 7.38-7.15 (m, 7H), 6.94 (m, 2H), 5.67 (m, 1H), 5.18-4.91 (m, 3H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 4.0-3.69 (m, 6H), 3.57 (broad s, 1H), 3.19-2.8 (m, 7H), 1.87 (m, 1H), 1.69-1.48 (m, 5H), 1.25 (m, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.3, 15.2.

Example 34

Monophospholactate 35: A solution of 34 (0.60 g, 0.71 mmol) and morpholine (0.31 mL, 3.54 mmol) in EtOAc (8 mL) was treated with HOAc (0.16 mL, 2.83 mmol) and NaBH<sub>3</sub>CN



(89 mg, 1.42 mmol). The reaction mixture was stirred at room temperature for 4 h. The product was partitioned between EtOAc and H<sub>2</sub>O. The organic phase was washed with brine, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (6% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (0.46 g, 70%, GS 278115, 1:1 diastereomeric mixture) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.74 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 7.38-7.15 (m, 7H), 6.92 (m, 2H), 5.66 (m, 1H), 5.2-5.0 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 3.97-3.57 (m, 12H), 3.2-2.78 (m, 7H), 2.46 (broad, s, 4H), 1.87 (m, 1H), 1.64-1.5 (m, 5H), 1.25 (m, 3H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.3, 15.3.

#### Example 35

Monophospholactate 37: A solution of 25 (0.50 g, 0.64 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2.0 mL) at 0°C was treated with trifluoroacetic acid (1 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3 mL) and cooled to 0°C. Triethylamine (0.45 mL, 3.20 mmol) was added followed by the treatment of 4-benzyloxybenzenesulfonyl chloride (0.18 g, 0.64 mmol, prepared according to Toja, E. et al. Eur. J. Med. Chem. 1991, 26, 403). The solution was stirred for 30 min at 0°C and then warmed to room temperature for 30 min. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (0.51 g, 85%) as a white solid.

#### Example 36

Monophospholactate 38: To a solution of 37 (0.48 g, 0.52 mmol) in EtOH (15 mL) was added 10% Pd/C (0.10 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and the crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (0.38 g, 88%, GS 273838, 1:1 diastereomeric mixture) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.86 (dd, 1H), 7.42-7.25 (m, 9H), 6.91 (m, 4H), 5.73 (d, J = 5.1 Hz, 1H), 5.42 (m, 1H), 5.18 (m, 2H), 4.76-4.31 (m,

2H), 4.22 (m, 2H), 4.12-3.75 (m, 6H), 3.63 (broad, s, 1H), 3.13 (m, 3H), 2.87 (m, 1H), 2.63 (m, 1H), 2.4 (m, 1H), 2.05 (m, 2H), 1.9 (m, 1H), 1.8 (m, 1H), 1.6 (m, 3H), 1.25 (m, 3H), 0.95 (d, J = 6.6 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.1, 15.7.

Example 37

Monophospholactate 40: A solution of 25 (0.75 g, 0.96 mmol) in  $\text{CH}_2\text{Cl}_2$  (2.0 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (1 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (4 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.67 mL, 4.80 mmol) was added followed by the treatment of 4-(4'-benzyloxycarbonyl piperazinyl)benzenesulfonyl chloride (0.48 g, 1.22 mmol, prepared according to Toja, E. et al. *Arzneim. Forsch.* 1994, 44, 501). The solution was stirred at  $0^\circ\text{C}$  for 1 h and then warmed to room temperature for 30 min. The product was partitioned between 10% 2-propanol/ $\text{CH}_2\text{Cl}_2$  and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.63 g, 60%) as a white solid.

Example 38

Monophospholactate 41: To a solution of 40 (0.62 g, 0.60 mmol) in MeOH (8 mL) and EtOAc (2 mL) was added 10% Pd/C (0.20 g). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was treated with 1.2 equivalent of TFA, co-evaporated with  $\text{CHCl}_3$  and dried under vacuum to give the monophospholactate (0.55 g, 90%) as a white solid.

Example 39

Monophospholactate 42: A solution of 41 (0.54 g, 0.53 mmol) and formaldehyde (0.16 mL, 5.30 mmol) in EtOAc (10 mL) was treated with HOAc (0.30 mL, 5.30 mmol) and  $\text{NaBH}_3\text{CN}$  (0.33 g, 5.30 mmol). The reaction mixture was stirred at room temperature overnight. The product was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The organic phase was washed with brine,

dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (6% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (97.2 mg, 20%, GS 277937, 1:1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.64 (d,  $J$  = 9.0 Hz, 2H), 7.38-7.17 (m, 7H), 6.95-6.88 (m, 4H), 5.67 (m, 1H), 5.2-4.96 (m, 2H), 4.57-4.4 (m, 2H), 4.2 (m, 2H), 3.97-3.64 (m, 8H), 3.49-3.37 (m, 4H), 3.05-2.78 (m, 12H), 1.88-1.62 (m, 3H), 1.58 (m, 3H), 1.25 (m, 3H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.3.

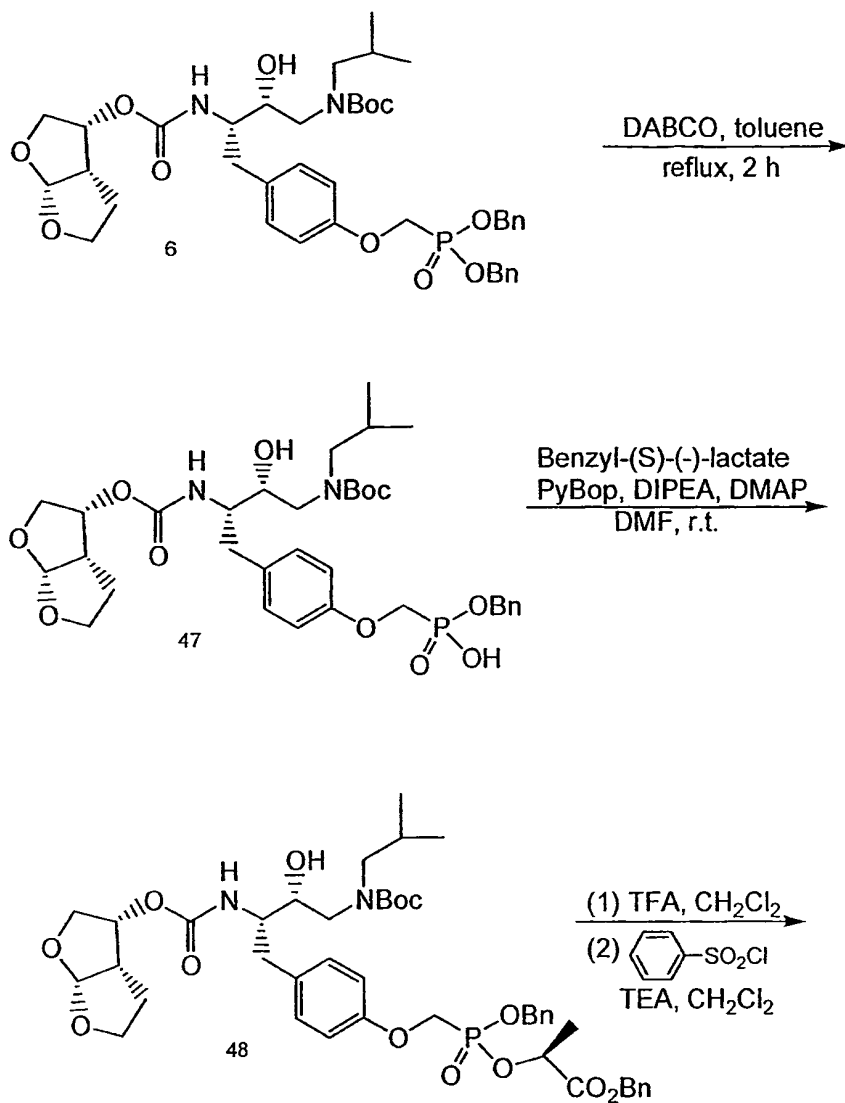
#### Example 40

Monophospholactate 45: A solution of 43 (0.12 g, 0.16 mmol) and lactate 44 (0.22 g, 1.02 mmol) in pyridine (1 mL) was heated to  $70^\circ\text{C}$  and 1,3-dicyclohexylcarbodiimide (0.17 g, 0.83 mmol) was added. The reaction mixture was stirred at  $70^\circ\text{C}$  for 4 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl,  $\text{H}_2\text{O}$ , saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (45 mg, 26%) as a white solid.

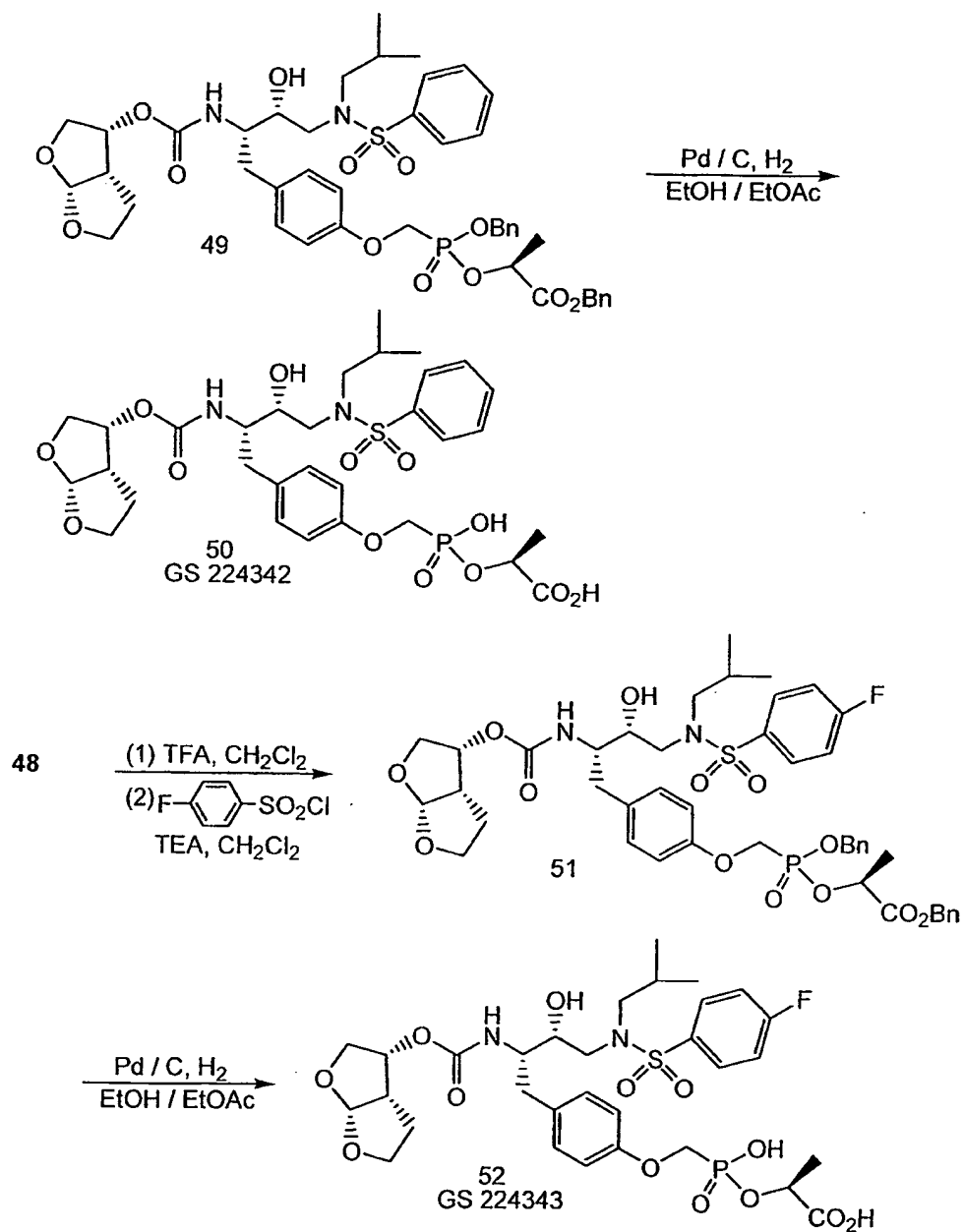
#### Example 41

Alcohol 46: To a solution of 45 (40 mg, 0.042 mmol) in EtOAc (2 mL) was added 20%  $\text{Pd}(\text{OH})_2/\text{C}$  (10 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 3 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and the product was dried under vacuum to give the alcohol (33 mg, 90%, GS 278809, 3/2 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J$  = 8.7 Hz, 2H), 7.39-7.15 (m, 7H), 7.02-6.88 (m, 4H), 5.66 (d,  $J$  = 4.5 Hz, 1H), 5.13-5.02 (m, 2H), 4.54-4.10 (m, 4H), 4.00-3.69 (m, 11H), 3.14 (m, 1H), 3.02-2.77 (m, 6H), 1.85-1.6 (m, 6H), 0.94 (d,  $J$  = 6.3 Hz, 3H), 0.89 (d,  $J$  = 6.3 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4, 15.9.

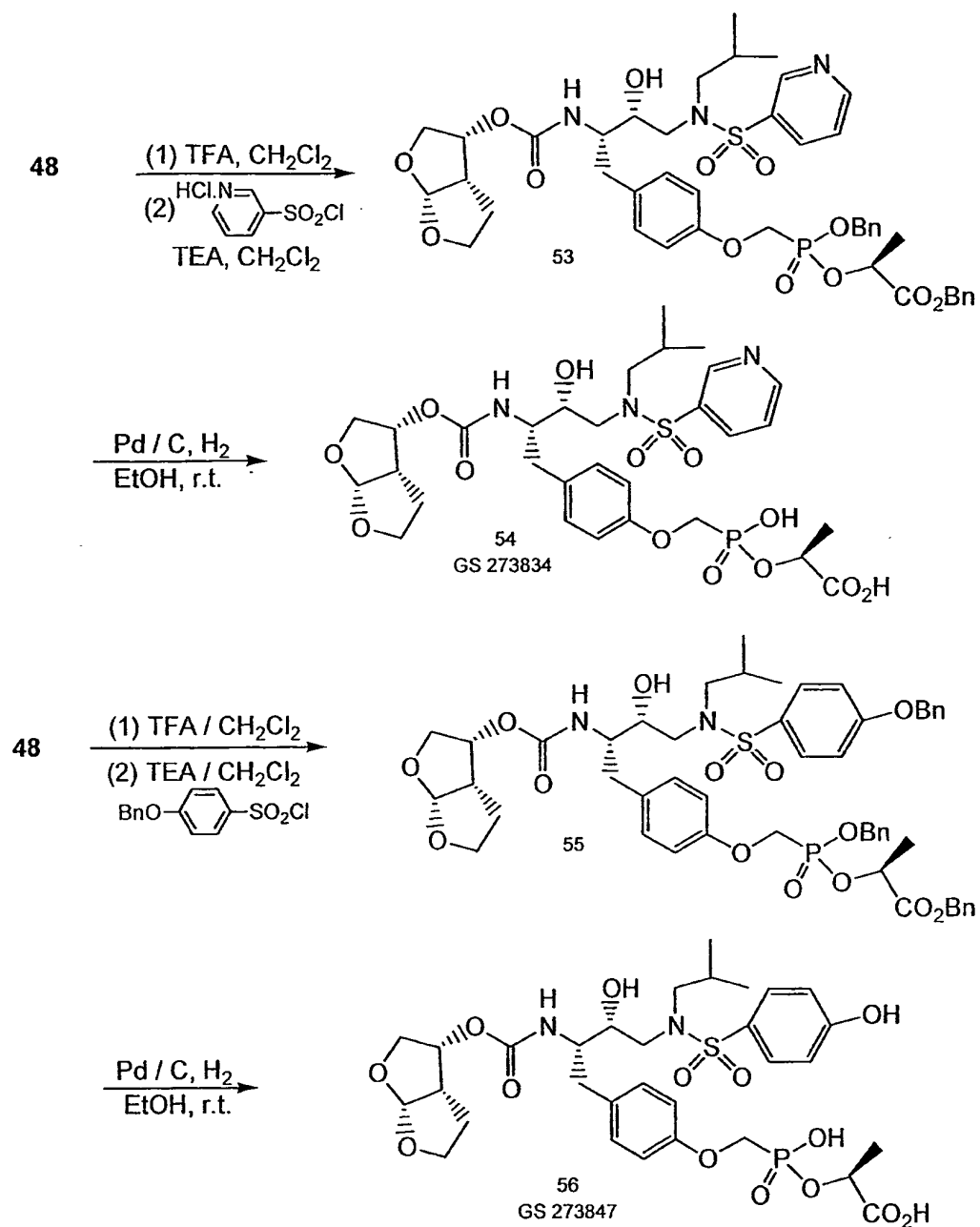
Scheme 12



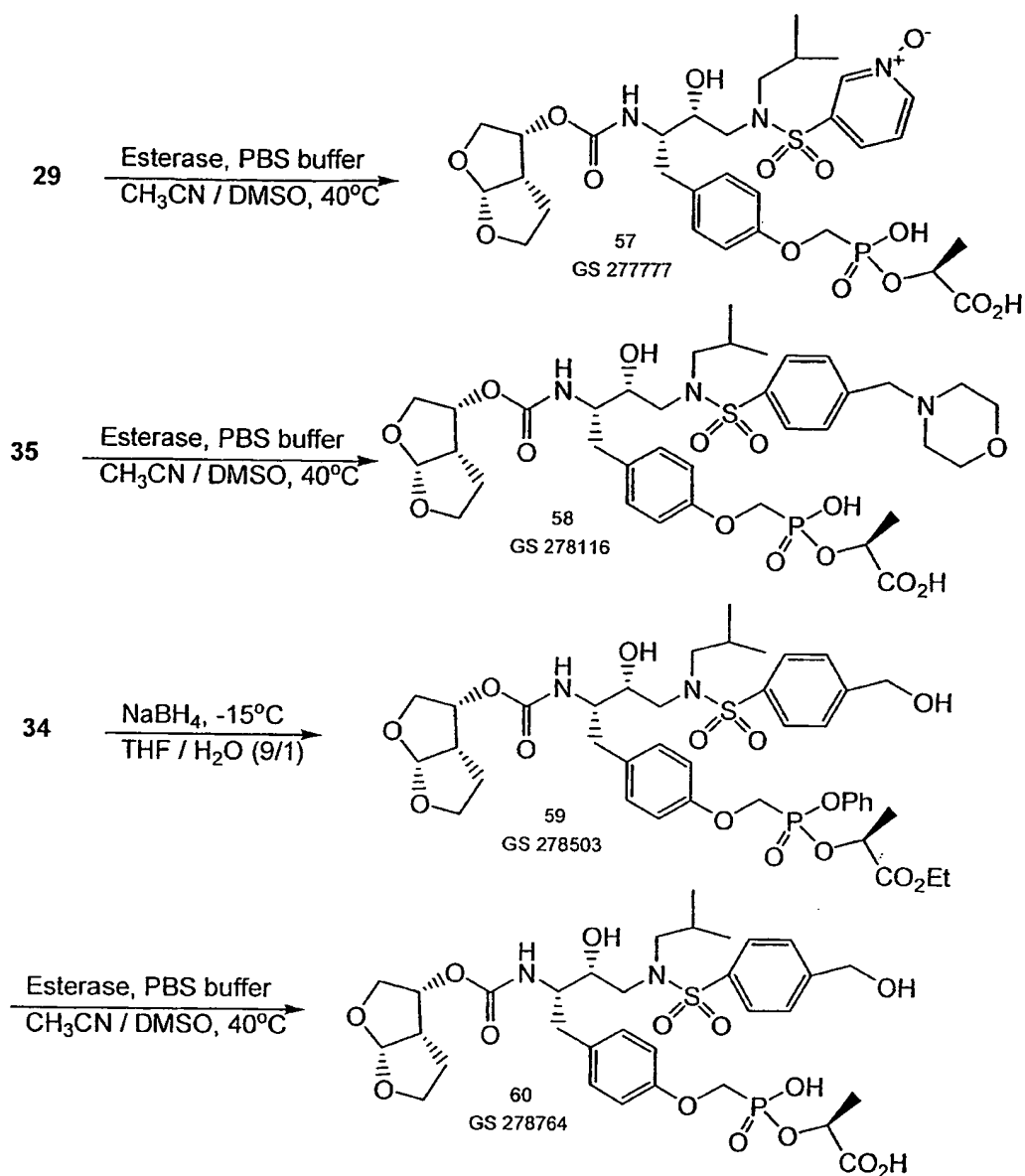
Scheme 13



## Scheme 14



## Scheme 15

Example 42

Monobenzylphosphonate 47: A solution of 6 (2.00 g, 2.55 mmol) and DABCO (0.29 g, 2.55 mmol) in toluene (10 mL) was heated to reflux for 2 h. The solvent was evaporated under reduced pressure. The residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with H<sub>2</sub>O, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated.

The crude product was dried under vacuum to give the monobenzylphosphonate (1.68 g, 95%) as a white solid.

#### Example 43

Monophospholactate 48: To a solution of 47 (2.5 g, 3.61 mmol) and benzyl-(S)-(-)-lactate (0.87 mL, 5.42 mmol) in DMF (12 mL) was added PyBop (2.82 g, 5.42 mmol) and *N,N*-diisopropylethylamine (2.51 mL, 14.44 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated. The residue was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with H<sub>2</sub>O, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (1.58 g, 51%) as a white solid.

#### Example 44

Monophospholactate 49: A solution of 48 (0.30 g, 0.35 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (0.6 mL) at 0°C was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and cooled to 0°C. Triethylamine (0.20 mL, 1.40 mmol) was added followed by the treatment of benzenesulfonyl chloride (62 mg, 0.35 mmol). The solution was stirred at 0°C for 30 min and then warmed to room temperature for 30 min. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (0.17 g, 53%) as a white solid.

#### Example 45

Metabolite X 50: To a solution of 49 (80 mg, 0.09 mmol) in EtOH (6 mL) and EtOAc (2 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 8 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl<sub>3</sub> and dried under vacuum to give the metabolite X (61 mg, 95%, GS 224342) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.83 (d, J = 6.9 Hz, 2H), 7.65-7.58 (m, 3H), 7.18 (d, J = 7.8 Hz, 2H), 6.90 (d, J = 7.8 Hz, 2H), 5.59



(d,  $J = 4.8$  Hz, 1H), 5.0 (m, 1H), 4.27 (d,  $J = 10.2$  Hz, 2H), 3.95-3.68 (m, 6H), 3.45 (dd, 1H), 3.18-2.84 (m, 6H), 2.50 (m, 1H), 2.02 (m, 1H), 1.6-1.38 (m, 5H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ ),  $\delta$  18.0.

i Example 46

Monophospholactate 51: A solution of 48 (0.28 g, 0.33 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.6 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.18 mL, 1.32 mmol) was added followed by the treatment of 4-fluorobenzenesulfonyl chloride (64 mg, 0.33 mmol). The solution was stirred at  $0^\circ\text{C}$  for 30 min and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.16 g, 52%) as a white solid.

Example 47

Metabolite X 52: To a solution of 51 (80 mg, 0.09 mmol) in EtOH (6 mL) and EtOAc (2 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 8 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with  $\text{CHCl}_3$  and dried under vacuum to give the metabolite X (61 mg, 95%, GS 224343) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.9 (dd, 2H), 7.32 (m, 2H), 7.18 (dd, 2H), 6.90 (dd, 2H), 5.59 (d,  $J = 5.4$  Hz, 1H), 5.0 (m, 1H), 4.28 (d,  $J = 10.2$  Hz, 2H), 3.95-3.72 (m, 6H), 3.44 (dd, 1H), 3.15-2.85 (m, 6H), 2.5 (m, 1H), 2.02 (m, 1H), 1.55-1.38 (m, 5H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.88 (d,  $J = 6.3$  Hz, 3H).  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  18.2.

Example 48

Monophospholactate 53: A solution of 48 (0.20 g, 0.24 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.6 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.3 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and

then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.16 mL, 1.20 mmol) was added followed by the treatment of hydrogen chloride salt of 3-pyridinsulfonyl chloride (50 mg, 0.24 mmol). The solution was stirred at  $0^\circ\text{C}$  for 30 min and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.11 g, 53%) as a white solid.

#### Example 49

Metabolite X 54: To a solution of 53 (70 mg, 0.09 mmol) in EtOH (5 mL) was added 10% Pd/C (20 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 5 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with  $\text{CHCl}_3$  and dried under vacuum to give the metabolite X (53 mg, 95%, GS 273834) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.99 (s, 1H), 8.79 (d,  $J$  = 4.2 Hz, 1H), 8.29 (d,  $J$  = 7.5 Hz, 1H), 7.7 (m, 1H), 7.15 (d,  $J$  = 8.4 Hz, 2H), 6.9 (d,  $J$  = 7.8 Hz, 2H), 5.59 (d,  $J$  = 5.4 Hz, 1H), 5.0 (m, 1H), 4.28 (d,  $J$  = 9.9 Hz, 2H), 3.97-3.70 (m, 6H), 3.44 (dd, 1H), 3.17-2.85 (m, 6H), 2.5 (m, 1H), 2.03 (m, 1H), 1.65-1.38 (m, 5H), 0.93 (d,  $J$  = 6.3 Hz, 3H), 0.88 (d,  $J$  = 6.3 Hz, 3H).  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  17.8.

#### Example 50

Monophospholactate 55: A solution of 48 (0.15 g, 0.18 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.12 mL, 0.88 mmol) was added followed by the treatment of 4-benzyloxybenzenesulfonyl chloride (50 mg, 0.18 mmol). The solution was stirred at  $0^\circ\text{C}$  for 30 min and then warmed to room temperature for 30 min. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.1 N HCl. The organic phase was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and

concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (0.11 g, 63%) as a white solid.

#### Example 51

Metabolite X 56: To a solution of 55 (70 mg, 0.07 mmol) in EtOH (4 mL) was added 10% Pd/C (20 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated, co-evaporated with CHCl<sub>3</sub> and dried under vacuum to give the metabolite X (46 mg, 90%, GS 273847) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD), δ 7.91 (s, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.1 Hz, 2H), 6.91 (m, 4H), 5.59 (d, J = 5.1 Hz, 1H), 5.0 (m, 1H), 4.27 (d, J = 10.2 Hz, 2H), 3.97-3.74 (m, 6H), 3.4 (dd, 1H), 3.17-2.8 (m, 6H), 2.5 (m, 1H), 2.0 (m, 1H), 1.6-1.38 (m, 5H), 0.93 (d, J = 6.3 Hz, 3H), 0.88 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 17.9.

#### Example 52

Metabolite X 57: To a suspension of 29 (40 mg, 0.05 mmol) in CH<sub>3</sub>CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (200 μL). The suspension was heated to 40°C for 48 h. The reaction mixture was concentrated, suspended in MeOH and filtered. The filtrate was concentrated and purified by HPLC to give the metabolite X (20 mg, 57%, GS 277777) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.68 (s, 1H), 8.47 (d, J = 6.0 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.68 (m, 1H), 7.15 (d, J = 8.4 Hz, 2H), 6.9 (d, J = 8.4 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.0 (m, 1H), 4.23 (d, J = 10.5 Hz, 2H), 3.97-3.68 (m, 6H), 3.45 (dd, 1H), 3.15-2.87 (m, 6H), 2.46 (m, 1H), 2.0 (m, 1H), 1.6-1.38 (m, 5H), 0.95 (d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 17.2.

#### Example 53

Metabolite X 58: To a suspension of 35 (60 mg, 0.07 mmol) in CH<sub>3</sub>CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (400 μL). The suspension was heated to 40°C for 3 days. The reaction mixture was concentrated, suspended in MeOH and filtered. The filtrate was concentrated and purified by HPLC to give the metabolite X (20 mg, 38%, GS 278116) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.74 (d, J = 6.9 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.1 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.0 (m,

2H), 4.41 (m, 2H), 4.22 (m, 2H), 3.97-3.65 (m, 12H), 3.15-2.9 (m, 8H), 2.75 (m, 1H), 2.0 (m, 1H), 1.8 (m, 2H), 1.53 (d, J = 6.9 Hz, 3H), 0.88 (m, 6H).

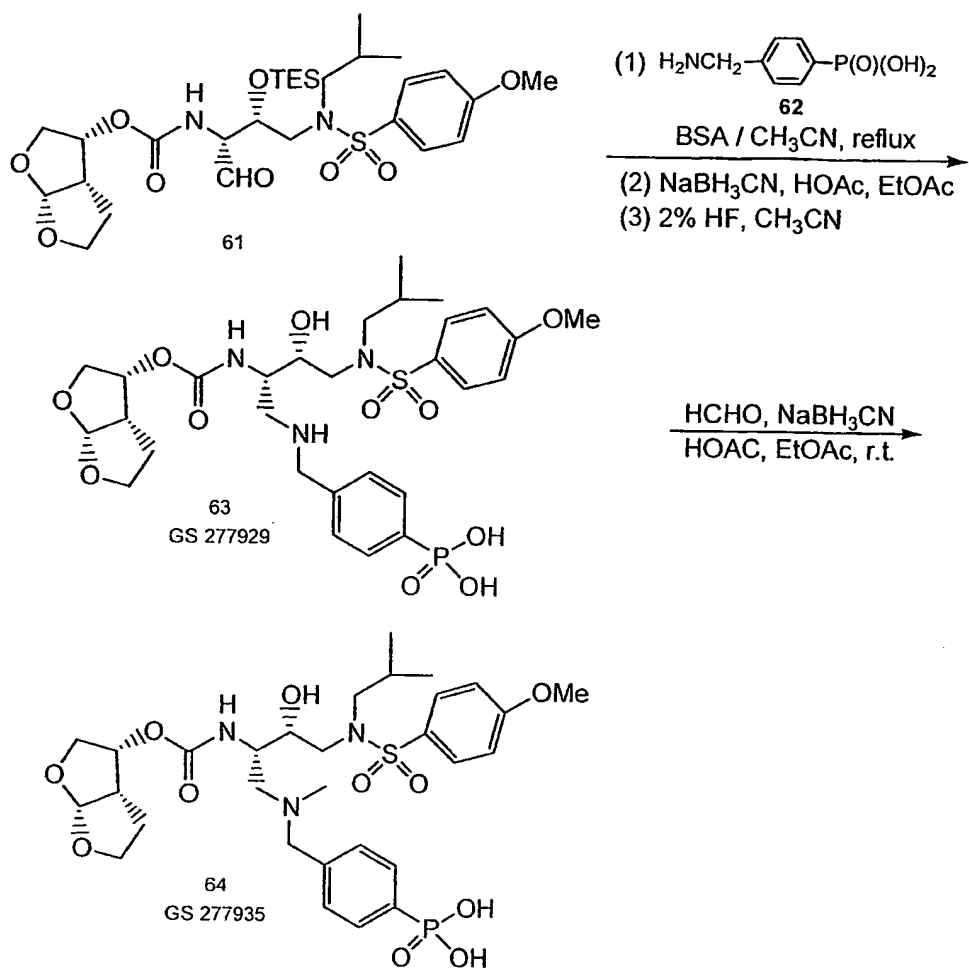
#### Example 54

- i Monophospholactate 59: A solution of 34 (2.10 g, 2.48 mmol) in THF (72 mL) and H<sub>2</sub>O (8 mL) at -15°C was treated with NaBH<sub>4</sub> (0.24 g, 6.20 mmol). The reaction mixture was stirred for 10 min at -15°C. The reaction was quenched with 5% aqueous NaHSO<sub>3</sub> and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 x). The combined organic layers were washed with H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give monophospholactate (1.89 g, 90%, GS 278053, 1:1 diastereomeric mixture) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.64 (m, 2H), 7.51 (m, 2H), 7.38-7.19 (m, 7H), 6.92 (m, 2H), 5.69 (d, J = 4.8 Hz, 1H), 5.15 (m, 2H), 4.76 (s, 2H), 4.54 (d, J = 10.5 Hz, 1H), 4.44 (m, 1H), 4.2 (m, 2H), 4.04-3.68 (m, 6H), 3.06-2.62 (m, 7H), 1.8 (m, 3H), 1.62-1.5 (dd, 3H), 1.25 (m, 3H), 0.94 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.4, 15.4.

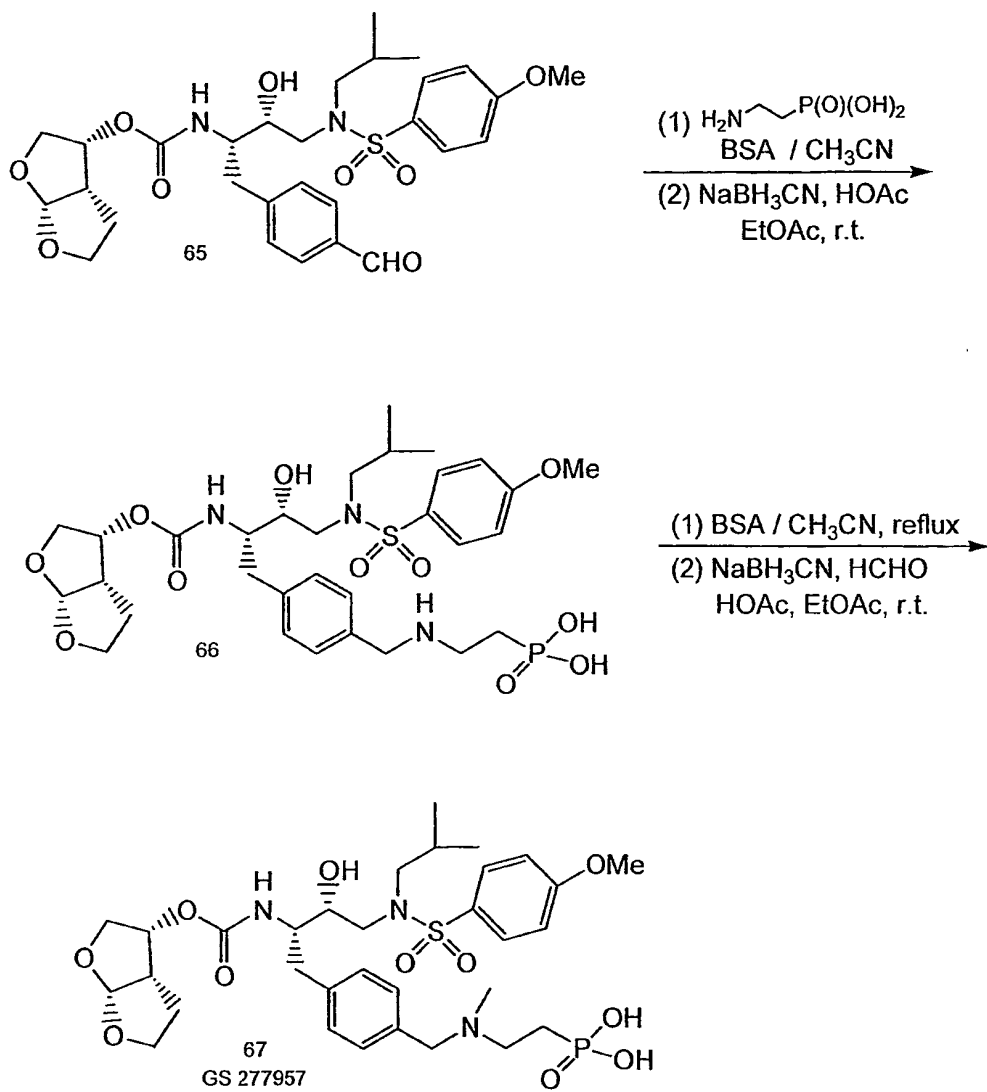
#### Example 55

Metabolite X 60: To a suspension of 59 (70 mg, 0.08 mmol) in CH<sub>3</sub>CN (1 mL), DMSO (0.5 mL), and 1.0 M PBS buffer (5 mL) was added esterase (600 µL). The suspension was heated to 40°C for 36 h. The reaction mixture was concentrated, suspended in MeOH and filtered. The filtrate was concentrated and purified by HPLC to give the metabolite X (22 mg, 36%, GS 278764) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.78 (dd, 2H), 7.54 (dd, 2H), 7.15 (m, 2H), 6.9 (m, 2H), 5.57 (d, 1H), 5.0 (m, 2H), 4.65 (m, 4H), 4.2 (m, 2H), 3.9-3.53 (m, 6H), 3.06-2.82 (m, 6H), 2.5 (m, 1H), 2.0 (m, 2H), 1.62-1.35 (m, 3H), 0.94 (m, 6H).

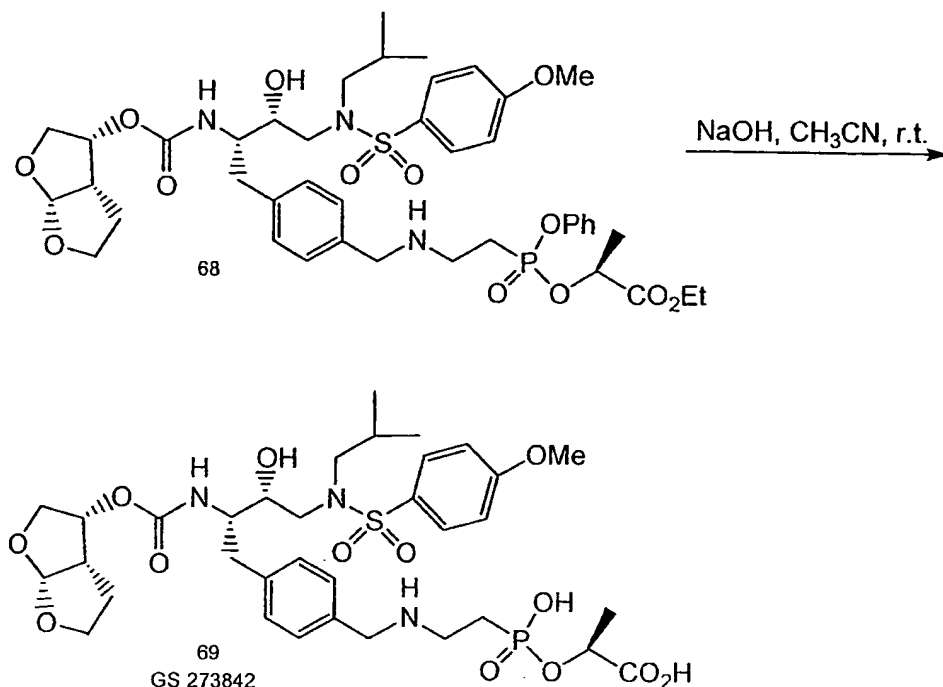
## Scheme 16



Scheme 17



## Scheme 18



## Example 56

Phosphonic Acid 63: Compound 62 (0.30 g, 1.12 mmol) was dissolved in CH<sub>3</sub>CN (5 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 2.2 mL, 8.96 mmol) was added. The reaction mixture was heated to reflux for 2 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (4 mL) and cooled to 0°C. Aldehyde 61 (0.20 g, 0.33 mmol), AcOH (0.18 mL, 3.30 mmol), and NaBH<sub>3</sub>CN (0.20 g, 3.30 mmol) were added. The reaction mixture was warmed to room temperature and stirred overnight. The reaction was quenched with H<sub>2</sub>O, stirred for 30 min, filtered, and concentrated. The crude product was dissolved in CH<sub>3</sub>CN (13 mL) and 48% aqueous HF (0.5 mL) was added. The reaction mixture was stirred at room temperature for 2 h and concentrated. The crude product was purified by HPLC to give the phosphonic acid (70 mg, 32%, GS 277929) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.92 (dd, 2H), 7.73 (d, *J* = 8.7 Hz, 2H), 7.63 (dd, 2H), 7.12 (d, *J* = 8.7 Hz, 2H), 5.68 (d, *J* = 5.1 Hz, 1H), 5.13 (m, 1H), 4.4 (m, 2H), 4.05-3.89 (m, 8H), 3.75 (m, 1H), 3.5 (m, 1H), 3.37 (m, 1H), 3.23-3.0 (m, 3H), 2.88-2.7 (m, 2H), 2.2 (m, 1H), 1.8 (m, 2H), 0.92 (d, *J* = 6.3 Hz, 3H), 0.85 (d, *J* = 6.3 Hz, 3H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 14.5.

### Example 57

Phosphonic Acid 64: A solution of 63 (50 mg, 0.07 mmol) and formaldehyde (60 mg, 0.70 mmol) in EtOAc (2 mL) was treated with HOAc (43  $\mu$ L, 0.70 mmol) and NaBH<sub>3</sub>CN (47 mg, 0.7 mmol). The reaction mixture was stirred at room temperature for 26 h. The reaction was quenched with H<sub>2</sub>O, stirred for 20 min, and concentrated. The crude product was purified by HPLC to give the phosphonic acid (15 mg, 29%, GS 277935) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  7.93 (m, 2H), 7.75 (m, 2H), 7.62 (m, 2H), 7.11 (m, 2H), 5.66 (m, 1H), 5.13 (m, 1H), 4.4 (m, 2H), 4.05-3.89 (m, 8H), 3.75 (m, 2H), 3.09-2.71 (m, 6H), 2.2 (m, 1H), 1.9 (m, 5H), 0.92 (d, J = 6.3 Hz, 3H), 0.85 (d, J = 6.3 Hz, 3H); <sup>31</sup>P NMR (CD<sub>3</sub>OD)  $\delta$  14.0.

### Example 58

Phosphonic Acid 66: 2-Aminoethylphosphonic acid (2.60 g, 21.66 mmol) was dissolved in CH<sub>3</sub>CN (40 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 40 mL) was added. The reaction mixture was heated to reflux for 2 h and cooled to room temperature and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (40 mL). Aldehyde 65 (1.33 g, 2.25 mmol), AcOH (1.30 mL, 22.5 mmol) and NaBH<sub>3</sub>CN (1.42 g, 22.5 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H<sub>2</sub>O, stirred for 1 h, filtered, and concentrated. The residue was dissolved in MeOH and filtered. The crude product was purified by HPLC to give the phosphonic acid (1.00 g, 63%) as a white solid.

### Example 59

Phosphonic Acid 67: Phosphonic acid 66 (0.13 g, 0.19 mmol) was dissolved in CH<sub>3</sub>CN (4 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.45 mL, 1.90 mmol) was added. The reaction mixture was heated to reflux for 2 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in EtOAc (3 mL). Formaldehyde (0.15 mL, 1.90 mmol), AcOH (0.11 mL, 1.90 mmol) and NaBH<sub>3</sub>CN (63 mg, 1.90 mmol) were added. The reaction mixture was stirred at room temperature overnight. The reaction was quenched with H<sub>2</sub>O, stirred for 6 h, filtered, and concentrated. The residue was dissolved in MeOH and filtered. The crude product was purified by HPLC to give the phosphonic acid (40 mg, 30%, GS

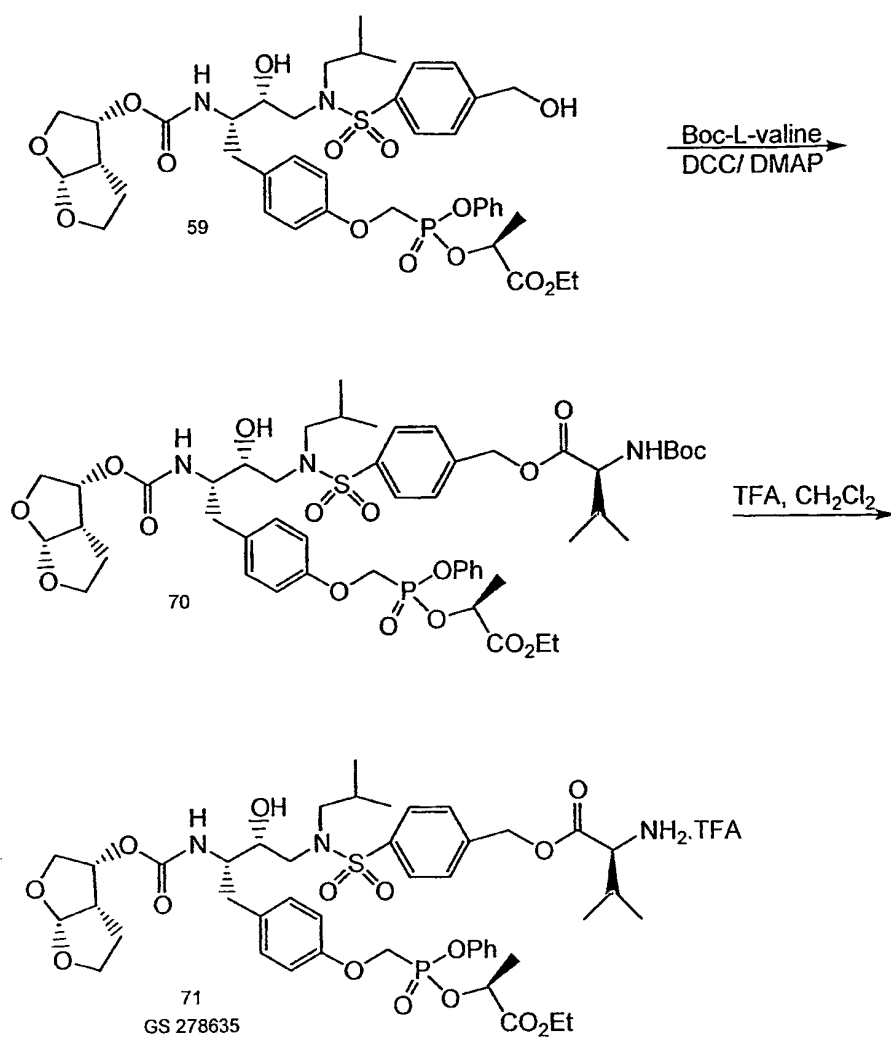


277957) as a white solid:  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.78 (d,  $J = 8.4$  Hz, 2H), 7.4 (m, 4H), 7.09 (d,  $J = 8.4$  Hz, 2H), 5.6 (d,  $J = 5.1$  Hz, 1H), 4.33 (m, 2H), 3.95-3.65 (m, 9H), 3.5-3.05 (m, 6H), 2.91-2.6 (m, 7H), 2.0 (m, 3H), 1.5 (m, 2H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.87 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  19.7.

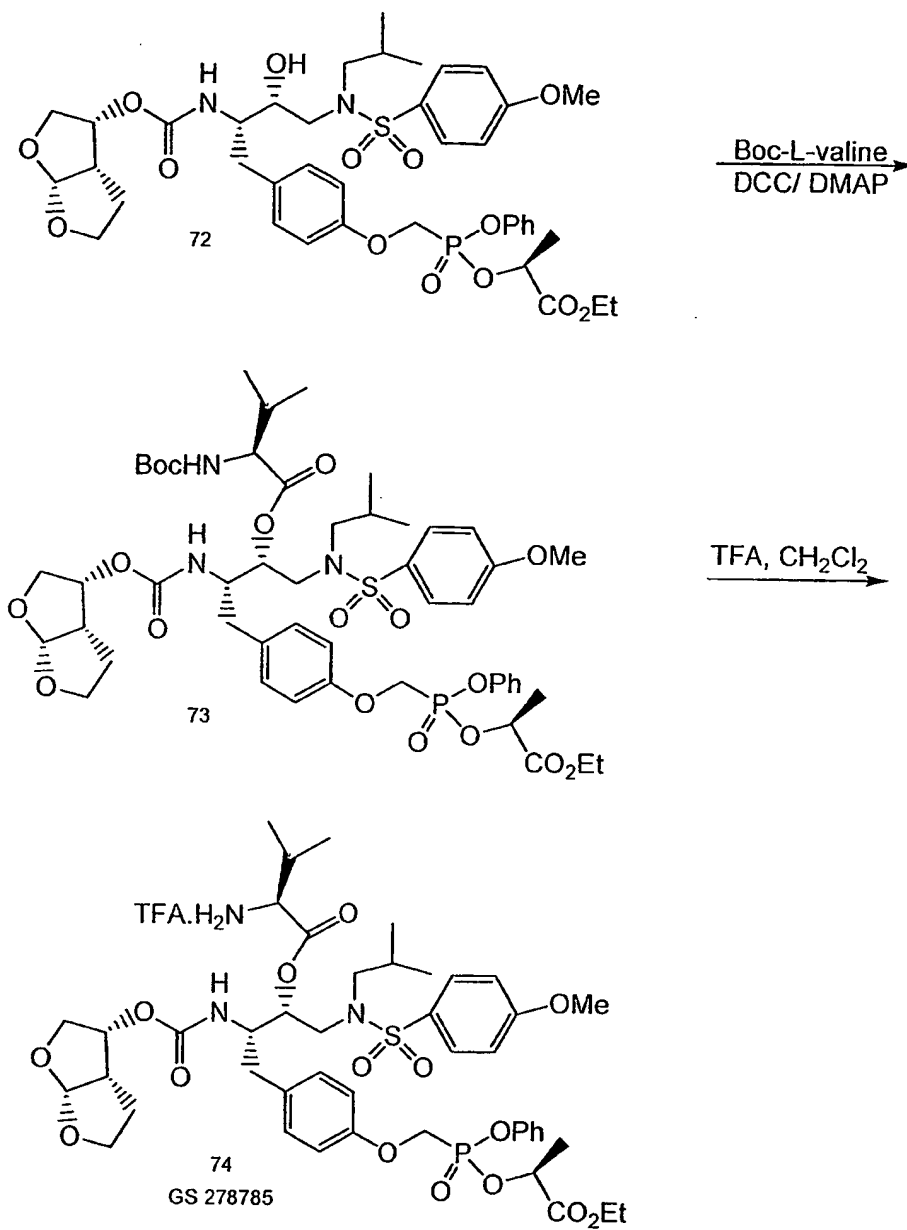
#### Example 60

Metabolite X 69: Monophospholactate 68 (1.4 g, 1.60 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (20 mL) and  $\text{H}_2\text{O}$  (20 mL). 1.0 N NaOH (3.20 mL, 3.20 mmol) was added. The reaction mixture was stirred at room temperature for 1.5 h and cooled to  $0^\circ\text{C}$ . The reaction mixture was acidified to  $\text{pH} = 1-2$  with 2 N HCl (1.6 mL, 3.20 mmol). The solvent was evaporated under reduced pressure. The crude product was purified by HPLC to give the metabolite X (0.60 g, 49%, GS 273842) as a white solid:  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  7.72 (d,  $J = 8.7$  Hz, 2H), 7.33 (m, 4H), 7.09 (d,  $J = 9.0$  Hz, 2H), 5.52 (d,  $J = 5.7$  Hz, 1H), 5.1 (broad, s, 1H), 4.85 (m, 1H), 4.63 (m, 1H), 4.13 (m, 2H), 3.8 (m, 5H), 3.6 (m, 4H), 3.36 (m, 1H), 3.03 (m, 4H), 2.79 (m, 3H), 2.5 (m, 1H), 2.0 (m, 3H), 1.5-1.3 (m, 5H), 0.85 (d,  $J = 6.6$  Hz, 3H), 0.79 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  21.9.

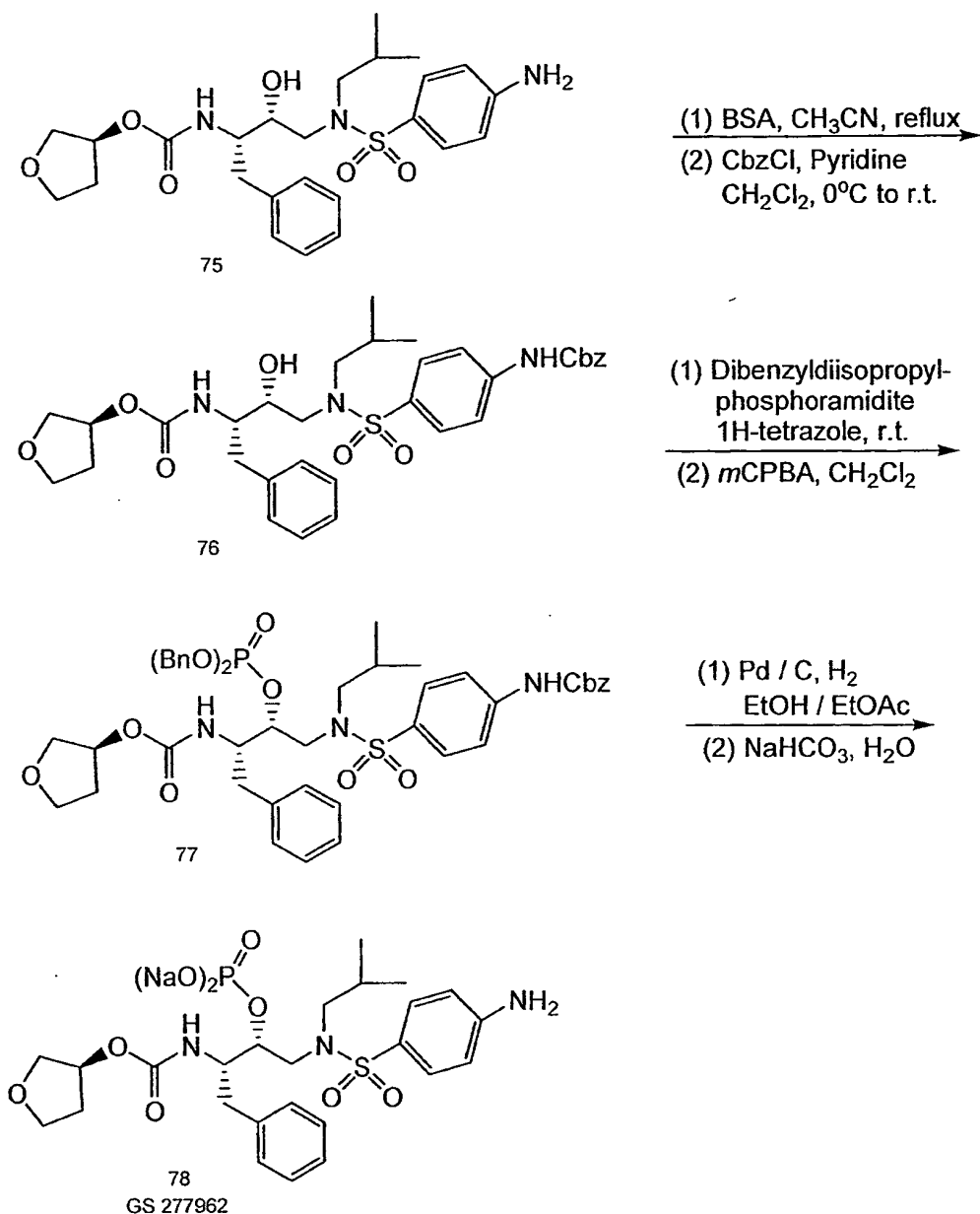
## Scheme 19



## Scheme 20



## Scheme 21



## Example 61

Monophospholactate 70: A solution of 59 (1.48 g, 1.74 mmol) and Boc-L-valine (0.38 g, 1.74 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) at 0°C was treated with 1,3- dicyclohexylcarbodiimide (0.45 g, 2.18 mmol) and 4-dimethylaminopyridine (26 mg, 0.21 mmol). The reaction mixture was stirred at 0°C for 1 h and then warmed to room temperature for 2 h. The product was

partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.2 N HCl. The organic layer was washed with  $\text{H}_2\text{O}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (1.65 g, 90%) as a white solid.

#### Example 62

Monophospholactate 71: A solution of 70 (1.65 g, 1.57 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (4 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (1.42 g, 85%, GS 278635, 2/3 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (m, 2H), 7.49 (d,  $J = 7.2$  Hz, 2H), 7.4-7.1 (m, 7H), 6.89 (m, 2H), 5.64 (m, 1H), 5.47 (m, 1H), 5.33-5.06 (m, 4H), 4.57-4.41 (m, 2H), 4.2 (m, 2H), 3.96-3.7 (m, 7H), 3.15-2.73 (m, 7H), 2.38 (m, 1H), 1.9 (m, 1H), 1.7 (m, 1H), 1.63-1.5 (m, 4H), 1.24 (m, 3H), 1.19 (m, 6H), 0.91 (d, 3H), 0.88 (d, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.4.

#### Example 63

Monophospholactate 73: A solution of 72 (0.43 g, 0.50 mmol) and Boc-L-valine (0.11 g, 0.50 mmol) in  $\text{CH}_2\text{Cl}_2$  (6 mL) was treated with 1,3-dicyclohexylcarbodiimide (0.13 g, 0.63 mmol) and 4-dimethylaminopyridine (62 mg, 0.5 mmol). The reaction mixture was stirred at room temperature overnight. The product was partitioned between  $\text{CH}_2\text{Cl}_2$  and 0.2 N HCl. The organic layer was washed with  $\text{H}_2\text{O}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (2% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.45 g, 85%) as a white solid.

#### Example 64

Monophospholactate 74: A solution of 73 (0.44 g, 0.42 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) at  $0^\circ\text{C}$  was treated with trifluoroacetic acid (0.5 mL). The solution was stirred for 30 min at  $0^\circ\text{C}$  and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (10% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (0.40 g, 90%, GS 278785, 1:1 diastereomeric mixture) as a white solid:

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.69 (d,  $J = 8.4$  Hz, 2H), 7.34-7.2 (m, 7H), 6.98 (d,  $J = 8.4$  Hz, 2H), 6.88 (m, 2H), 6.16 (m, 1H), 5.64 (m, 1H), 5.46 (m, 1H), 5.2-5.0 (m, 2H), 4.5 (m, 2H), 4.2 (m, 3H), 4.0-3.4 (m, 9H), 3.3 (m, 1H), 3.0-2.8 (m, 5H), 2.5 (m, 1H), 1.83 (m, 1H), 1.6-1.5 (m, 5H), 1.25 (m, 3H), 1.15 (m, 6H), 0.82 (d,  $J = 6.0$  Hz, 3H), 0.76 (d,  $J = 6.0$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3, 15.5.

#### Example 65

Cbz Amide 76: Compound 75 (0.35 g, 0.69 mmol) was dissolved in  $\text{CH}_3\text{CN}$  (6 mL). *N,O*-Bis(trimethylsilyl)acetamide (BSA, 0.67 mL, 2.76 mmol) was added. The reaction mixture was heated to reflux for 1 h, cooled to room temperature, and concentrated. The residue was co-evaporated with toluene and chloroform and dried under vacuum to give a thick oil which was dissolved in  $\text{CH}_2\text{Cl}_2$  (3 mL) and cooled to  $0^\circ\text{C}$ . Pyridine (0.17 mL, 2.07 mmol) and benzyl chloroformate (0.12 mL, 0.83 mmol) were added. The reaction mixture was stirred at  $0^\circ\text{C}$  for 1 h and then warmed to room temperature overnight. The reaction was quenched with MeOH (5 mL) and 10% HCl (20 mL) at  $0^\circ\text{C}$  and stirred for 1 h. The product was extracted with  $\text{CH}_2\text{Cl}_2$ , washed with brine, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the CBz amide (0.40 g, 90%) as a white solid.

#### Example 66

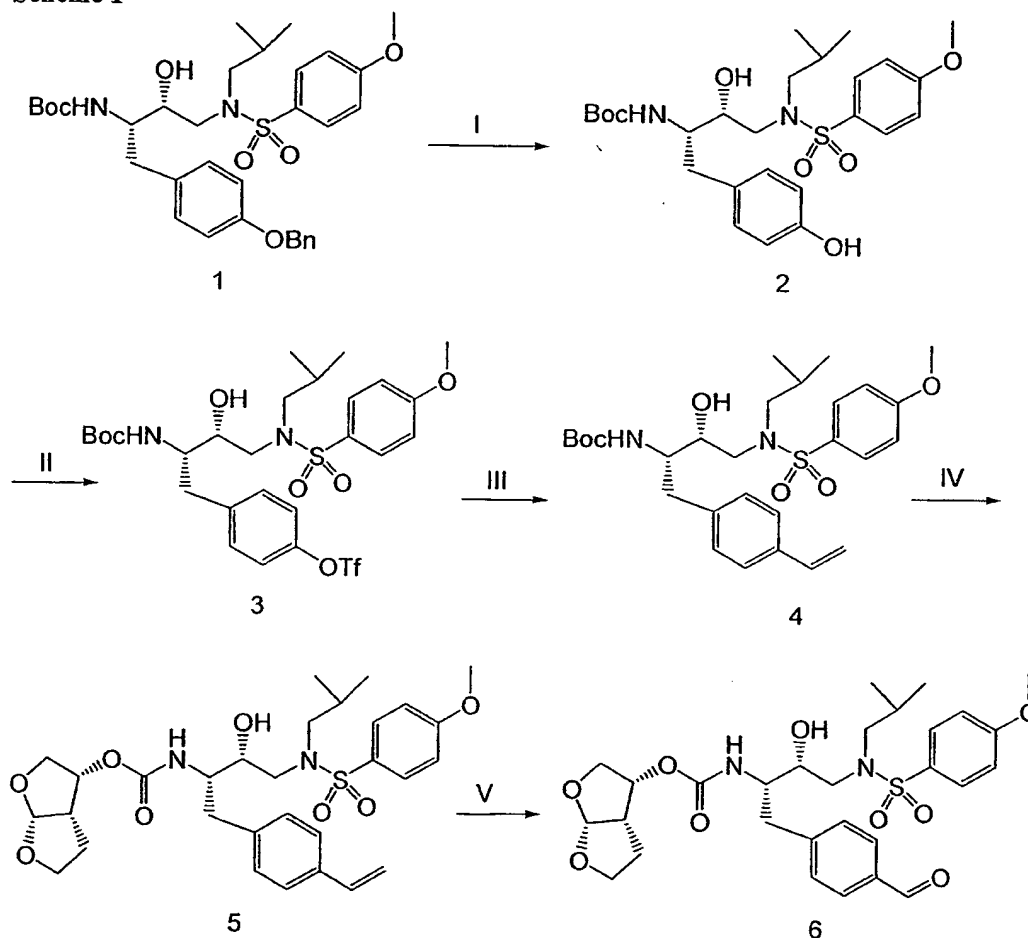
Dibenzylphosphonate 77: A solution of 76 (0.39 g, 0.61 mmol) and 1*H*-tetrazole (54 mg, 0.92 mmol) in  $\text{CH}_2\text{Cl}_2$  (8 mL) was treated with dibenzyl-diisopropylphosphoramidite (0.32 g, 0.92 mmol) and stirred at room temperature overnight. The solution was cooled to  $0^\circ\text{C}$ , treated with *m*CPBA, stirred for 1 h at  $0^\circ\text{C}$  and then warmed to room temperature for 1 h. The reaction mixture was poured into a mixture of aqueous  $\text{Na}_2\text{SO}_3$  and  $\text{NaHCO}_3$  and extracted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with  $\text{H}_2\text{O}$ , dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the dibenzylphosphonate (0.42 g, 76%) as a white solid.

#### Example 67

Disodium Salt of Phosphonic Acid 78: To a solution of 77 (0.18 g, 0.20 mmol) in EtOH (20 mL) and EtOAc (4 mL) was added 10% Pd/C (40 mg). The suspension was stirred under  $\text{H}_2$  atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through

a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (0.11 g, 95%) which was dissolved in H<sub>2</sub>O (4 mL) and treated with NaHCO<sub>3</sub> (32 mg, 0.38 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (0.12 g, 99%, GS 277962) as a white solid: <sup>1</sup>H NMR (D<sub>2</sub>O) δ 7.55 (dd, 2H), 7.2 (m, 5H), 7.77 (dd, 2H), 4.65 (m, 1H), 4.24 (m, 1H), 4.07 (m, 1H), 3.78-2.6 (m, 12H), 1.88-1.6 (m, 3H), 0.75 (m, 6H).

Scheme 1



I. H<sub>2</sub>/10% Pd-C/EtOAc-EtOH ; II. Tf<sub>2</sub>NPh/Cs<sub>2</sub>CO<sub>3</sub>;  
 III. Bu<sub>3</sub>SnCH=CH<sub>2</sub>/PdCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>/LiCl/DMF/90 C;  
 IV. a. TFA/CH<sub>2</sub>Cl<sub>2</sub>; b. Bisfurancarboxylate/*i*-Pr<sub>2</sub>NEt/DMAP;  
 V. NaIO<sub>4</sub>/OsO<sub>4</sub>/EtOAc-H<sub>2</sub>O

Example 1

Compound 1 was prepared by methods from Examples herein.

Example 2

Compound 2: To a solution of compound 1 (47.3 g) in EtOH/EtOAc (1000 mL/500 mL) was added 10% Pd-C (5 g). The mixture was hydrogenated for 19 hours. Celite was added and the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and was washed with ethyl acetate. Concentration gave compound 2 (42.1 g).

Example 3

Compound 3: To a solution of compound 2 (42.3 g, 81 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (833 mL) was added N-phenyltrifluoromethanesulfonimide (31.8 g, 89 mmol), followed by cesium carbonate (28.9 g, 89 mmol). The mixture was stirred for 24 hours. The solvent was removed under reduced pressure, and ethyl acetate was added. The reaction mixture was washed with water (3x) and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/EtOAc = 13/1) gave compound 3 (49.5 g) as a white powder.

Example 4

Compound 4: To a solution of compound 3 (25.2, 38.5 mmol) in DMF (240 mL) was added lithium chloride (11.45 g, 270 mmol), followed by dichlorobis(triphenylphosphine) palladium(II) (540 mg, 0.77 mmol). The mixture was stirred for 3 minutes under high vacuum and recharged with nitrogen. To the above solution was added tributylvinyltin (11.25 mL). The reaction mixture was heated at 90°C for 6 hours and cooled to 25°C. Water was added to the reaction, and the mixture was extracted with ethyl acetate (3X). The combined organic layer was washed with water (6x) and brine, and dried over MgSO<sub>4</sub>. Concentration gave an oil. The oil was diluted with dichloromethane (40 mL), water (0.693 mL, 38.5 mmol) and DBU (5.76 mL, 38.5 mmol) were added. The mixture was stirred for 5 minutes, and subjected to flash column chromatography (hexanes/EtOAc = 2.5/1). Compound 4 was obtained as white solid (18.4 g).



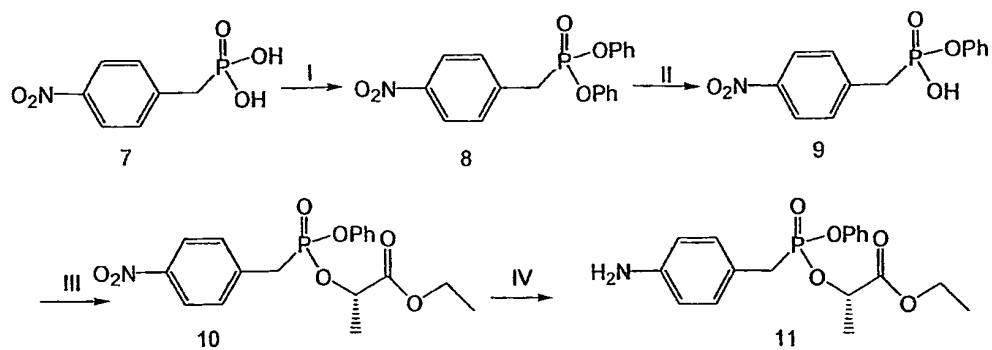
Example 5

Compound 5: To a solution of compound 4 (18.4 g, 34.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (70 mL) at  $0^\circ\text{C}$  was added trifluoroacetic acid (35 mL). The mixture was stirred at  $0^\circ\text{C}$  for 2 hrs, and solvents were evaporated under reduced pressure. The reaction mixture was quenched with saturated sodium carbonate solution, and was extracted with ethyl acetate (3x). The combined organic layer was washed with saturated sodium carbonate solution (1x), water (2x), and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration gave a solid. To a solution of the above solid in acetonitrile (220 mL) at  $0^\circ\text{C}$  was added bisfurancarboxylic acid (10.09 g, 34.2 mmol), followed by di-isopropylethylamine (12.0 mL, 69.1 mmol) and DMAP (843 mg, 6.9 mmol). The mixture was warmed to  $25^\circ\text{C}$  and stirred for 12 hours. Solvents were removed under reduced pressure. The mixture was diluted with ethyl acetate, and was washed with water (2X), 5% hydrochloric acid (2x), water (2x), 1N sodium hydroxide (2x), water (2x), and brine (1x), and dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 1/1) gave compound 5 (13.5 g).

Example 6

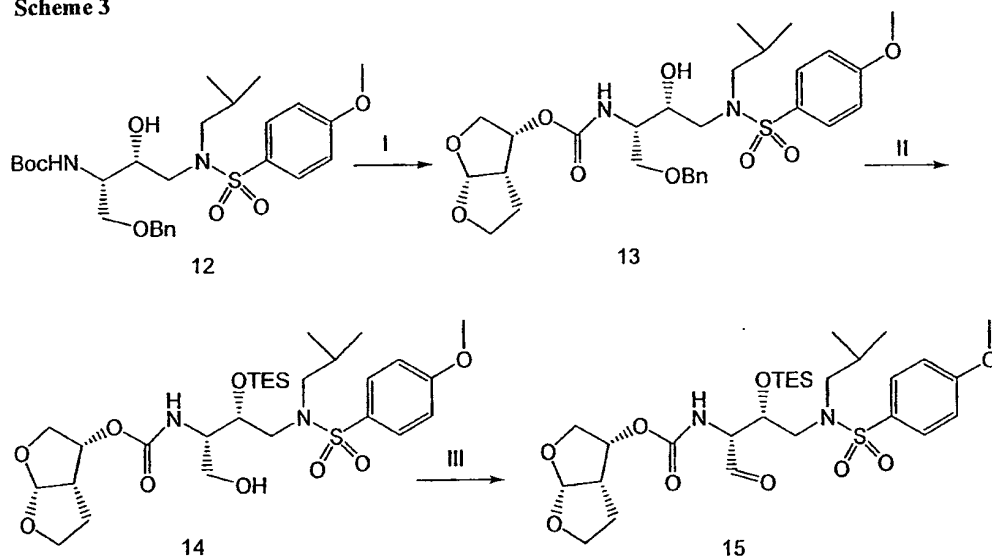
Compound 6: To a solution of compound 5 (13.5 g, 23 mmol) in ethyl acetate (135 mL) was added water (135 mL), followed by 2.5% osmium tetroxide/tert-butanol (17 mL). Sodium periodate (11.5 g) was added in portions over 2 minutes period. The mixture was stirred for 90 minutes, and was diluted with ethyl acetate. The organic layer was separated and washed with water (3x) and brine (1x), and dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 1/2) gave compound 6 as white powder (12 g):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.98 (1 H, s), 7.82 (2 H, m), 7.75 (2 H, m), 7.43 (2 H, m), 6.99 (2 H, m), 5.64 (1 H, m), 5.02 (2 H, m), 4.0-3.8 (9 H, m), 3.2-2.7 (7 H, m), 1.9-1.4 (3 H, m), 0.94 (6 H, m).

Scheme 2



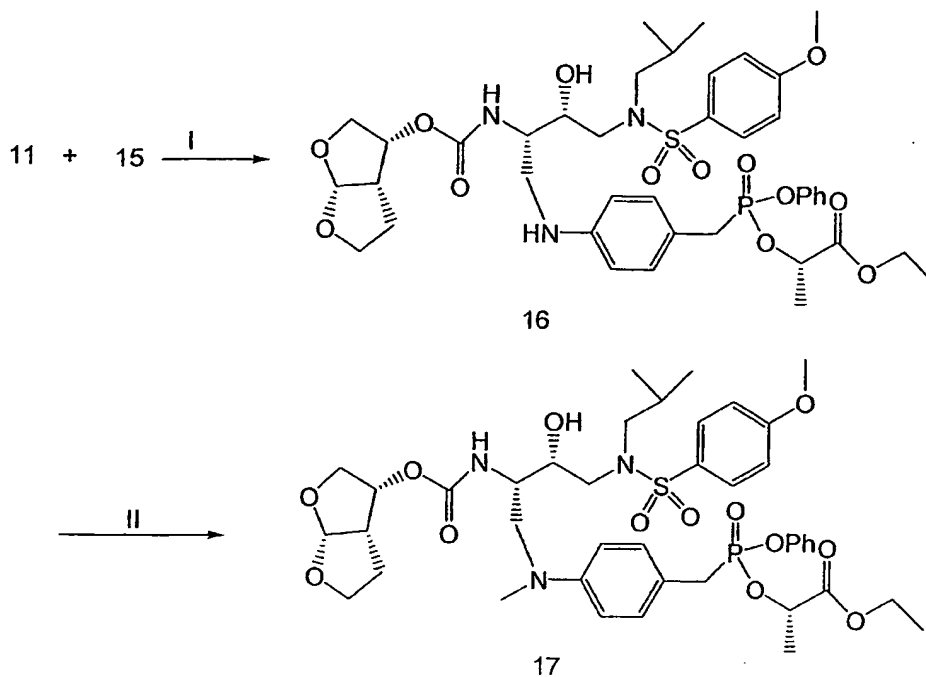
I. a.  $\text{SOCl}_2$ /toluene/60 °C; b. PhOH/pyridine; II. a. NaOH/THF/ $\text{H}_2\text{O}$ ; b. HCl;  
 III. b.  $\text{SOCl}_2$ /toluene/60 °C; c. ethyl lactate/pyridine; IV.  $\text{H}_2$ /10% Pd-C/EtOAc

Scheme 3



I. a. TFA/ $\text{CH}_2\text{Cl}_2$ ; b. bisfurancarboxylate/ $i\text{-Pr}_2\text{NEt}$ /DMAP; II. a.  $\text{Et}_3\text{SiCl}$ /imidazole/DMF;  
 b.  $\text{H}_2$ /20% Pd(OH) $_2$ -C/ $i\text{PrOH}$ ; III. Des-Martin reagent/ $\text{CH}_2\text{Cl}_2$

Scheme 4



I. a.  $\text{NaBH}_3\text{CN}/\text{HOAc}/\text{EtOAc}$ ; b.  $2\%\text{HF}/\text{CH}_3\text{CN}$ ;  
 II.  $\text{HCHO}/\text{NaBH}_3\text{CN}/\text{HOAc}/\text{EtOAc}$

#### Example 8

Compound 8: To the suspension of compound 7 (15.8 g, 72.5 mmol) in toluene (140 mL) was added DMF (1.9 mL), followed by thionyl chloride (53 mL, 725 mmol). The reaction mixture was heated at  $60^\circ\text{C}$  for 5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x), EtOAc, and  $\text{CH}_2\text{Cl}_2$  (2x) to afford a brown solid. To the solution of the brown solid in  $\text{CH}_2\text{Cl}_2$  at  $0^\circ\text{C}$  was added phenol (27.2 g, 290 mmol), followed by slow addition of pyridine (35 mL, 435 mmol). The reaction mixture was allowed to warm to  $25^\circ\text{C}$  and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration gave a dark oil, which was purified by flash column chromatography (hexanes/EtOAc = 4/1 to 1/1) to afford compound 8 (12.5 g).

Example 9

Compound 9: To a solution of compound 8 (2.21 g, 6 mmol) in THF (30 mL) was added 12 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 2 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and acetic acid (343 mL, 6 mmol) was added. The aqueous phase was washed with EtOAc (3x), and then acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave compound 9 as a solid (1.1 g).

Example 10

Compound 10: To a suspension of compound 9 (380 mg, 1.3 mmol) in toluene (2.5 mL) was added thionyl chloride (1 mL, 13 mmol), followed by DMF (1 drop). The mixture was heated at 60°C for 2 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) and CH<sub>2</sub>Cl<sub>2</sub> to give a white solid. To the solution of the above solid in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) at -20°C was added ethyl lactate (294 µL, 2.6 mmol), followed by pyridine (420 µL, 5.2 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure to give a yellow solid, which was purified by flash column chromatography to generate compound 10 (427 mg).

Example 11

Compound 11: To a solution of compound 10 (480 mg) in EtOAc (20 mL) was added 10% Pd-C (80 mg). The reaction mixture was hydrogenated for 6 hrs. The mixture was stirred with celite for 5 mins, and filtered through a pad of celite. Concentration under reduced pressure gave compound 11 (460 mg).

Example 12

Compound 12 was prepared by the methods of the Examples herein

Example 13

Compound 13: To a solution of compound 12 (536 mg, 1.0 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added trifluoroacetic acid (2 mL). The mixture was stirred for 2 hrs, and was concentrated under reduced pressure. The liquid was coevaporated with CH<sub>2</sub>Cl<sub>2</sub> (3x) and EtOAc (3x) to

give a brown solid. To the solution of above brown solid in acetonitrile (6.5 mL) at 0°C was added bisfurancarboxylate (295 mg, 1.0 mmol), followed by diisopropylethylamine (350 µL, 2.0 mmol) and DMAP (24 mg). The mixture was warmed to 25°C, and was stirred for 12 hrs. The mixture was diluted with EtOAc, and was washed sequentially with water (2x), 0.5 N HCl (2x), water (2x), 0.5 N NaOH solution (2x), water (2x), and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 1/1) afford compound 13 (540 mg).

#### Example 14

Compound 14: To a solution of compound 13 (400 mg, 0.67 mmol) in DMF (3 mL) was added imidazole (143 mg, 2.10 mmol), followed by triethylchlorosilane (224 µL, 1.34 mmol). The mixture was stirred for 12 hours. The mixture was diluted with EtOAc, and was washed with water (5x) and brine, and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave a white solid (427 mg). To the solution of above solid in isopropanol (18 mL) was added 20% palladium(II) hydroxide on carbon (120 mg). The mixture was hydrogenated for 12 hours. The mixture was stirred with celite for 5 mins, and filtered through a pad of celite. Concentration under reduced pressure gave compound 14 (360 mg).

#### Example 15

Compound 15: To a solution of compound 14 (101 mg, 0.18 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was added Dess-Martin periodinane (136 mg, 0.36 mmol). The mixture was stirred for 1 hour. Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave compound 15 (98 mg).

#### Example 16

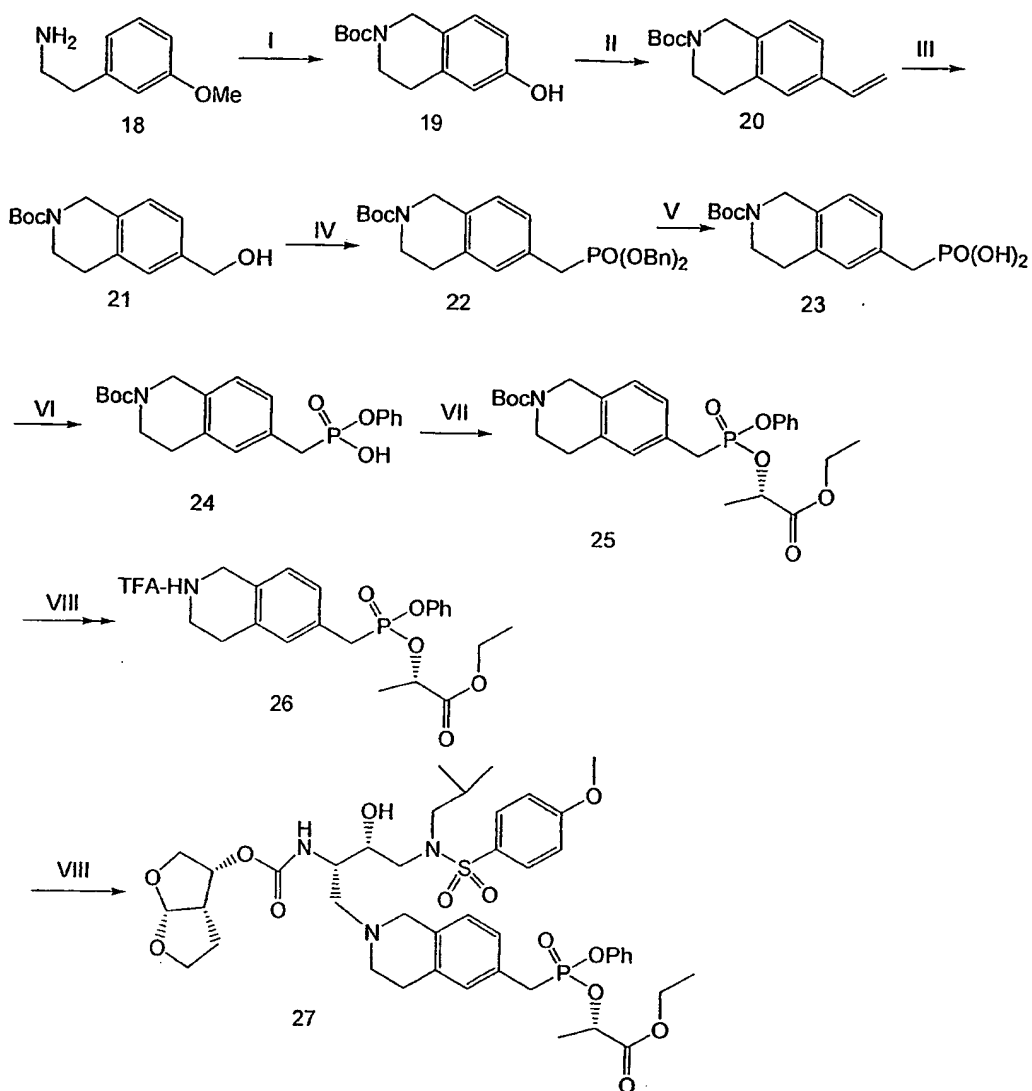
Compound 16: To a solution of compound 15 (50 mg, 0.08 mmol) in EtOAc (0.5 mL) was added compound 11 (150 mg, 0.41 mmol). The mixture was cooled to 0°C, acetic acid (19 µL, 0.32 mmol) was added, followed by sodium cyanoborohydride (10 mg, 0.16 mmol). The mixture was warmed to 25°C, and was stirred for 14 hrs. The mixture was diluted with EtOAc, and was washed with water (3x) and brine, and was dried over MgSO<sub>4</sub>. Concentration gave a oil. To the solution of above oil in acetonitrile (2.5 mL) was added 48% HF/CH<sub>3</sub>CN (0.1 mL). The mixture was stirred for 30 minutes, and was diluted with

EtOAc. The organic phase was washed with water (3x) and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/3) gave compound 16 (50 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (2 H, d, J = 8.9 Hz), 7.15-7.05 (7 H, m), 7.30 (2 H, d, J = 8.9 Hz), 6.64 (2 H, m), 5.73 (1 H, m), 5.45 (1 H, m), 5.13 (1 H, m), 4.93 (1 H, m), 4.22-3.75 (11 H, m), 3.4 (4 H, m), 3.35-2.80 (5 H, m), 2.1-1.8 (3 H, m), 1.40-1.25 (6 H, m), 0.94 (6 H, m).

#### Example 17

Compound 17: To a solution of compound 16 (30 mg, 0.04 mmol) in EtOAc (0.8 mL) was added 37% formaldehyde (26 μL, 0.4 mmol). The mixture was cooled to 0°C, acetic acid (20 μL, 0.4 mmol) was added, followed by sodium cyanoborohydride (22 mg, 0.4 mmol). The mixture was warmed to 25°C, and was stirred for 14 hrs. The mixture was diluted with EtOAc, and was washed with water (3x) and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/3) gave compound 17 (22 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.63 (2 H, m), 7.3-6.9 (9 H, m), 6.79 (2 H, m), 5.68 (1 H, m), 5.2 (1 H, m), 5.10 (1 H, m), 4.95 (1 H, m), 4.22 (2 H, m), 4.2-3.7 (21 H, m), 2.0-1.7 (3 H, m), 1.4-1.2 (6 H, m), 0.93 (6 H, m).

Scheme 5

**Example 18**

Compound 18: Compound 18 was purchased from Aldrich.

Example 19

Compound 19: To compound 18 (12.25 g, 81.1 mmol) was added 37% formaldehyde (6.15 mL, 82.7 mmol) slowly. The mixture was heated at 100°C for 1 hour. The mixture was cooled to 25°C, and was diluted with benzene, and was washed with water (2x).

- 5 Concentration under reduced pressure gave a yellow oil. To above oil was added 20% HCl (16 mL), and the mixture was heated at 100°C for 12 hours. The mixture was basified with 40% KOH solution at 0°C, and was extracted with EtOAc (3x). The combined organic layer was washed with water and brine, and was dried over MgSO<sub>4</sub>. Concentration gave a oil. To the oil was added 48% HBr (320 mL), and the mixture was heated at 120°C for 3 hours.
- 1 Water was removed at 100°C under reduced pressure to give a brown solid. To the solution of above solid in water/dioxane (200 mL/200mL) at 0°C was added sodium carbonate (25.7 g, 243 mmol) slowly, followed by di-tert-butyl dicarbonate (19.4 g, 89 mmol). The mixture was warmed to 25°C and stirred for 12 hours. Dioxane was removed under reduced pressure, and the remaining was extracted with EtOAc (3x). The combined organic phase was washed with water (3x) and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 4/1 to 3/1) gave compound 19 as white solid (13.6 g).

Example 20

Compound 20: To a solution of compound 19 (2.49 g, 10 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (100 mL) was added N-phenyltrifluoromethanesulfonimide (3.93 g, 11 mmol), followed by cesium carbonate (3.58 g, 11 mmol). The mixture was stirred for 48 hours. The solvent was removed under reduced pressure, and ethyl acetate was added. The reaction mixture was washed with water (3x) and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 6/1) gave a white solid (3.3 g). To the solution of above solid (2.7 g, 7.1 mmol) in DMF (40 mL) was added lithium chloride (2.11 g, 49.7 mmol), followed by dichlorobis(triphenylphosphine) palladium(II) (100 mg, 0.14 mmol). The mixture was stirred for 3 minutes under high vacuum and recharged with nitrogen. To the above solution was added tributylvinyltin (2.07 mL, 7.1 mmol). The reaction mixture was heated at 90°C for 3 hours and cooled to 25°C. Water was added to the reaction, and the mixture was extracted with ethyl acetate (3X). The combined organic layer was washed with water (6x) and brine, and dried over MgSO<sub>4</sub>. Concentration gave an oil. The oil was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL), water (128 µL, 7.1mmol) and DBU (1 mL, 7.1 mmol) were added. The



mixture was stirred for 5 minutes, and was subjected to flash column chromatography (hexanes/EtOAc = 9/1). Compound 20 was obtained as white solid (1.43 g).

#### Example 21

- i Compound 21: To a solution of compound 20 (1.36 g, 5.25 mmol) in ethyl acetate (16 mL) was added water (16 mL), followed by 2.5% osmium tetroxide/tert-butanol (2.63 mL). Sodium periodate (2.44 g) was added in portions over 2 minutes period. The mixture was stirred for 45 minutes, and was diluted with ethyl acetate. The organic layer was separated and washed with water (3x) and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration gave a brown solid. To the solution of above solid in methanol (100 mL) at  $0^\circ\text{C}$  was added sodium borohydride. The mixture was stirred for 1 hour at  $0^\circ\text{C}$ , and was quenched with saturated  $\text{NH}_4\text{Cl}$  (40 mL). Methanol was removed under reduced pressure, and the remaining was extracted with EtOAc (3x). The combined organic layer was washed with water and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 2/1) gave compound 21 (1.0 g).

#### Example 22

Compound 22: To a solution of compound 21 (657 mg, 2.57 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added a solution of tetrabromocarbon (1.276 g, 3.86 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL). To the above mixture was added a solution of triphenylphosphine (673 mg, 2.57 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) over 30 minutes period. The mixture was stirred for 2 hours, and was concentrated under reduced pressure. Purification by flash column chromatography (hexanes/EtOAc = 9/1) gave the bromide intermediate (549 mg). To the solution of above bromide (548 mg, 1.69 mmol) in acetonitrile (4.8 mL) was added dibenzyl phosphite (0.48 mL, 2.19 mmol), followed by cesium carbonate (828 mg, 2.54 mmol). The mixture was stirred for 48 hours, and was diluted with EtOAc.

The mixture was washed with water (3x) and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 3/1 to 100% EtOAc) gave compound 22 (863 mg).

#### Example 23

Compound 23: To a solution of compound 22 (840 mg) in ethanol (80 mL) was added 10% palladium on carbon (200 mg). The mixture was hydrogenated for 2 hours. The mixture was

stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 23 (504 mg).

#### Example 24

Compound 24: To a solution of compound 23 (504 mg, 1.54 mmol) in pyridine (10.5 mL) was added phenol (1.45 g, 15.4 mmol), followed by DCC (1.28 g, 6.2 mmol). The mixture was heated at 65°C for 3 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered and washed with EtOAc (2x5 mL). Concentration gave a oil, which was purified by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{isopropanol} = 100/3$ ) to give diphenylphosphonate intermediate (340 mg). To a solution of above compound (341 mg, 0.71 mmol) in THF (1 mL) was added 0.85 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 3 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x), and then acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave compound 24 as a solid (270 mg).

#### Example 25:

Compound 25: To a solution of compound 24 (230 mg, 0.57 mmol) in DMF (2 mL) was added ethyl (s)-lactate (130  $\mu\text{L}$ , 1.14 mmol), followed by diisopropylethylamine (400  $\mu\text{L}$ , 2.28 mmol) and benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (504 mg, 1.14 mmol). The mixture was stirred for 14 hours, was diluted with EtOAc. The organic phase was washed with water (5x) and brine (1x), and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{isopropanol} = 100/3$ ) gave compound 25 (220 mg).

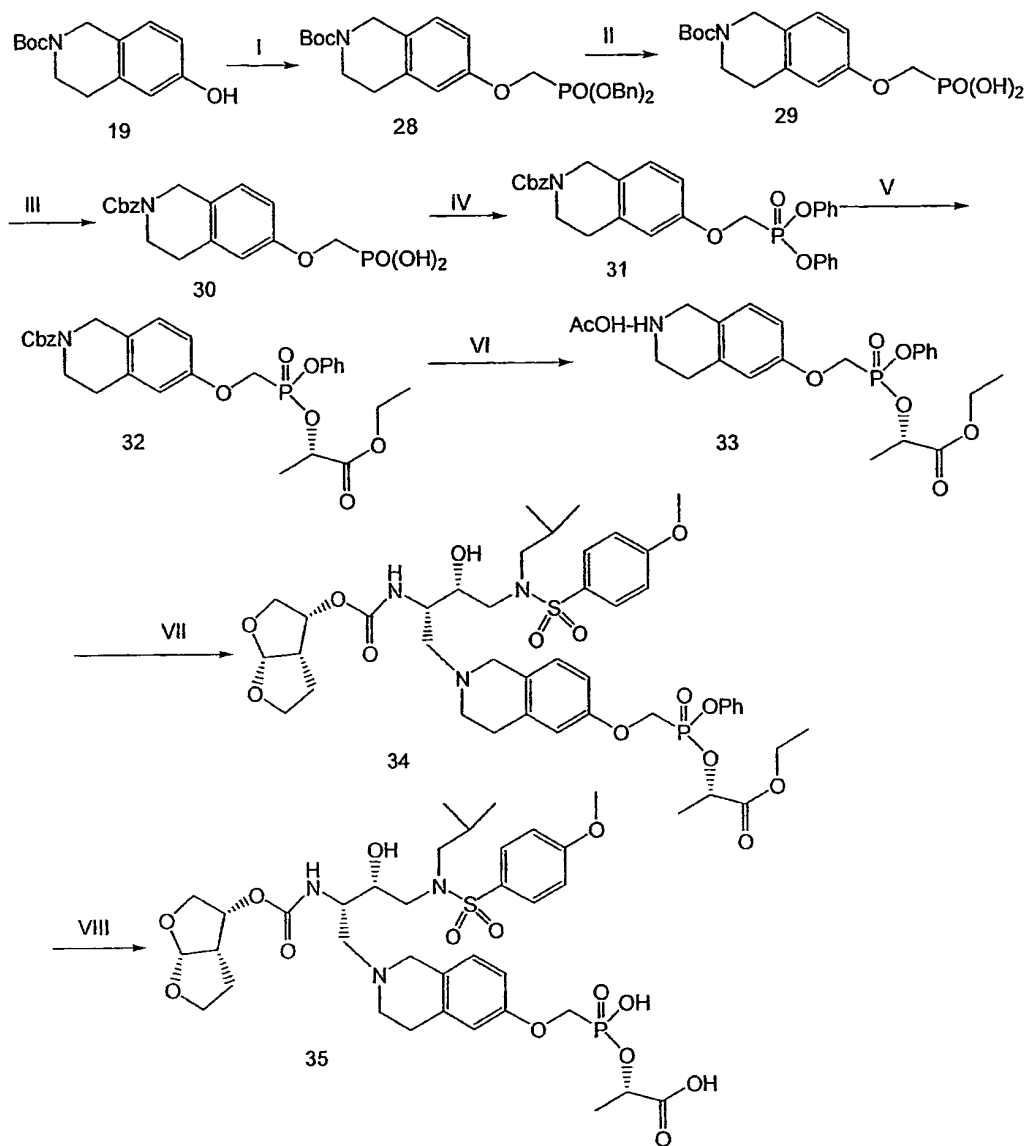
#### Example 26

Compound 26: To a solution of compound 25 (220 mg) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added trifluoroacetic acid (1 mL). The mixture was stirred for 2 hrs, and was concentrated under reduced pressure. The mixture was diluted with EtOAc, and was washed with saturated sodium carbonate solution, water, and brine, and was dried over  $\text{MgSO}_4$ . Concentration gave compound 26 (170 mg).

Example 27

Compound 27: To a solution of compound 15 (258 mg, 0.42 mmol) in EtOAc (2.6 mL) was added compound 26 (170 mg, 0.42 mmol), followed by acetic acid (75  $\mu$ L, 1.26 mmol). The mixture was stirred for 5 minutes, and sodium cyanoborohydride (53 mg, 0.84 mmol) was added. The mixture was stirred for 14 hrs. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/4$  to  $100/6$ ) gave the intermediate (440 mg). To the solution of above compound (440 mg) in acetonitrile (10 mL) was added 48% HF/  $\text{CH}_3\text{CN}$  (0.4 mL). The mixture was stirred for 2 hours, and acetonitrile was removed under reduced pressure. The remaining was diluted with EtOAc, and was washed with water (3x) and brine (1x), and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$ ) gave compound 27 (120 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.70 (2 H, m), 7.27 (2 H, m), 7.15 (5 H, m), 6.95 (3 H, m), 5.73 (1 H, m), 5.6-5.4 (1 H, m), 5.16 (1 H, m), 4.96 (1 H, m), 4.22-3.60 (13 H, m), 3.42 (2 H, m), 3.4-2.6 (11 H, m), 2.1-3.8 (3 H, m), 1.39 (3 H, m), 1.24 (3 H, m), 0.84 (6 H, m).

Scheme 6

**Example 28**

Compound 28: To a solution of compound 19 (7.5 g, 30 mmol) in acetonitrile (420 mL) was added dibenzyl triflate (17.8 g, 42 mmol), followed by cesium carbonate (29.4 g, 90 mmol).

The mixture was stirred for 2.5 hours, and was filtered. Acetonitrile was removed under reduced pressure, and the remaining was diluted with EtOAc. The mixture was washed with water (3x) and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 2/1 to 1/1) gave compound 28 (14.3 g).

#### Example 29

Compound 29: To a solution of compound 28 (14.3 g) in ethanol (500 mL) was added 10% palladium on carbon (1.45 g). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 29 (9.1 g).

#### Example 30

Compound 30: To a solution of compound 29 (9.1 g) in CH<sub>2</sub>Cl<sub>2</sub> (60 mL) was added trifluoroacetic acid (30 mL). The mixture was stirred for 4 hrs, and was concentrated under reduced pressure. The mixture was coevaporated with CH<sub>2</sub>Cl<sub>2</sub> (3x) and toluene, and was dried under high vacuum to give a white solid. The white solid was dissolved in 2.0 N NaOH solution (45 mL, 90 mmol), and was cooled to 0°C. To the above solution was added slowly a solution of benzyl chloroformate (6.4 mL, 45 mmol) in toluene (7 mL). The mixture was warmed to 25°C, and was stirred for 6 hours. 2.0 N sodium hydroxide was added to above solution until pH = 11. The aqueous was extracted with ethyl ether (3x), and was cooled to 0°C. To the above aqueous phase at 0°C was added concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combine organic layers were washed with brine, and were dried over MgSO<sub>4</sub>. Concentration gave compound 30 (11.3 g) as a white solid.

#### Example 31

Compound 31: To the suspension of compound 30 (11.3 g, 30 mmol) in toluene (150 mL) was added thionyl chloride (13 mL, 180 mmol), followed by DMF (a few drops). The reaction mixture was heated at 65°C for 4.5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x) to afford a brown solid. To the solution of the brown solid in CH<sub>2</sub>Cl<sub>2</sub> (120 mL) at 0°C was added phenol (11.28 g, 120 mmol), followed by slow addition of pyridine (14.6 mL, 180 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture

was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration gave a dark oil, which was purified by flash column chromatography (hexanes/EtOAc = 3/1 to 1/1) to afford compound 31 (9.8 g).

#### Example 32

Compound 32: To a solution of compound 31 (9.8 g, 18.5 mmol) in THF (26 mL) was added 20.3 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 2.5 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x). The aqueous phase was cooled to 0°C, and was acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over  $\text{MgSO}_4$ . Concentration under reduced pressure gave a solid (8.2 g). To a suspension of above solid (4.5 g, 10 mmol) in toluene (50 mL) was added thionyl chloride (4.4 mL, 60 mmol), followed by DMF (0.2 mL). The mixture was heated at 70°C for 3.5 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) to give a white solid. To the solution of the above solid in  $\text{CH}_2\text{Cl}_2$  (40 mL) at 0°C was added ethyl (s)-lactate (2.3 mL, 20 mmol), followed by pyridine (3.2 mL, 40 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was diluted with EtOAc. The organic phase was washed with 1 N HCl, water, and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 2/1 to 1/1) gave compound 32 (4.1 g).

#### Example 33

Compound 33: To a solution of compound 32 (3.8 g, 6.9 mmol) in EtOAc/EtOH (30 mL/30 mL) was added 10% palladium on carbon (380 mg), followed by acetic acid (400  $\mu\text{L}$ , 6.9 mmol). The mixture was hydrogenated for 3 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 33 (3.5 g).

#### Example 34

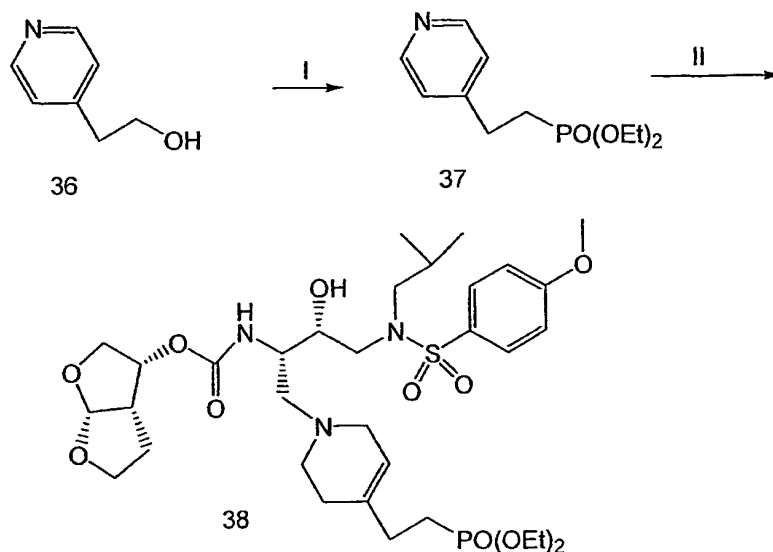
Compound 34: To a solution of compound 15 (1.70 g, 2.76 mmol) in EtOAc (17 mL) was added compound 33 (3.50 g, 6.9 mmol). The mixture was stirred for 5 minutes, and was cooled to 0°C, and sodium cyanoborohydride (347 mg, 5.52 mmol) was added. The mixture

was stirred for 6 hrs. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/6) gave the intermediate (3.4 g). To the solution of above compound (3.4 g) in acetonitrile (100 mL) was added 48% HF/ CH<sub>3</sub>CN (4 mL). The mixture was stirred for 2 hours, and acetonitrile was removed under reduced pressure. The remaining was diluted with EtOAc, and was washed with saturated sodium carbonate, water (3x), and brine (1x), and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/5) gave compound 34 (920 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (2 H, m), 7.38-7.19 (5 H, m), 6.92 (3 H, m), 6.75 (2 H, m), 5.73 (1 H, m), 5.57-5.35 (1 H, m), 5.16 (2 H, m), 4.5 (2 H, m), 4.2-3.6 (13 H, m), 3.25-2.50 (11 H, m), 2.0-1.8 (3 H, m), 1.5 (3 H, m), 1.23 (3 H, m), 0.89 (6 H, m).

#### Example 35

Compound 35: To a solution of compound 34 (40 mg) in CH<sub>3</sub>CN /DMSO (1 mL/0.5 mL) was added 1.0 M PBS buffer (5 mL), followed by esterase (200 µL). The mixture was heated at 40°C for 48 hours. The mixture was purified by reverse phase HPLC to give compound 35 (11 mg).

## Scheme 7



I. a.  $\text{SOCl}_2$ /toluene/60 °C; b.  $\text{P}(\text{OEt})_3$ /toluene/120 °C;  
II. a. compound 14/ $\text{Tf}_2\text{O}$ ; b.  $\text{NaBH}_4$ /EtOH/HOAc; c. 2% HF/ $\text{CH}_3\text{CN}$

Example 36

Compound 36: Compound 36 was purchased from Aldrich.

Example 37

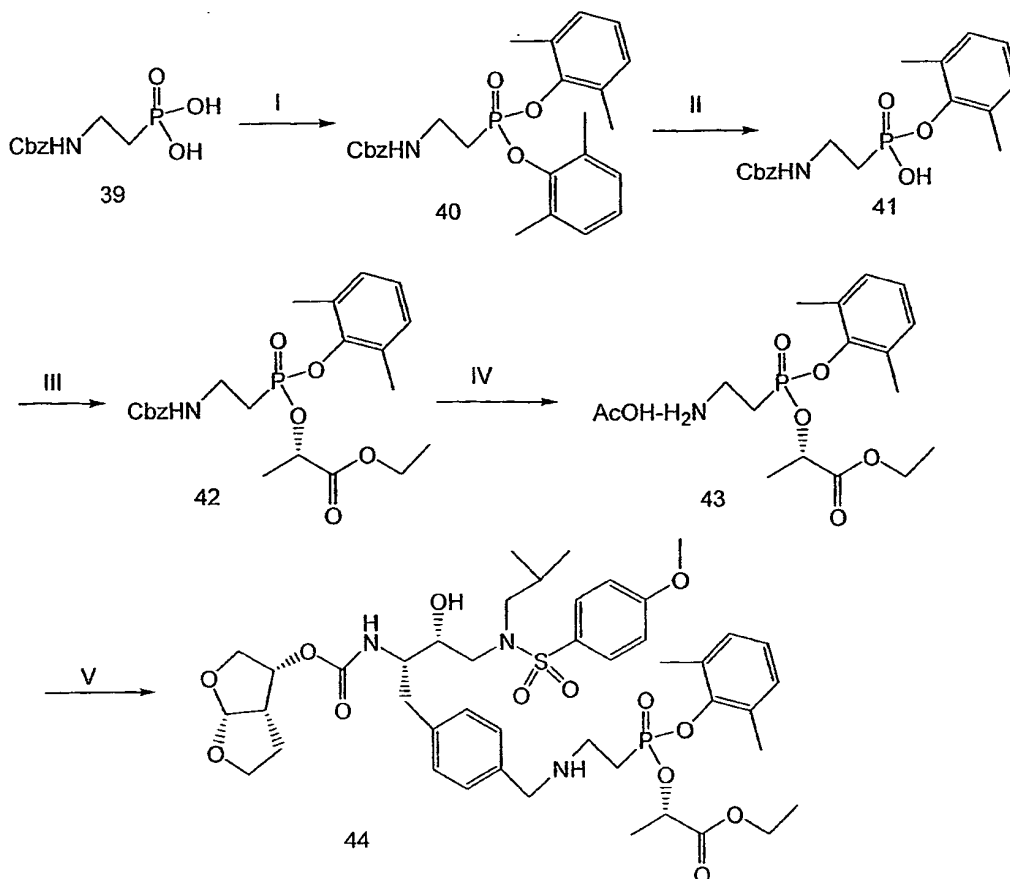
Compound 37: To a solution of compound 36 (5.0 g, 40 mmol) in chloroform (50 mL) was added thionyl chloride (12 mL) slowly. The mixture was heated at 60°C for 2.5 hours. The mixture was concentrated under reduced pressure to give a yellow solid. To the suspension of above solid (5.2 g, 37 mmol) in toluene (250 mL) was added triethyl phosphite (19 mL, 370 mmol). The mixture was heated at 120°C for 4 hours, and was concentrated under reduced pressure to give a brown solid. The solid was dissolved in EtOAc, and was basified with 1.0 N NaOH. The organic phase was separated and was washed with water (2x) and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 9/1$ ) gave compound 37 (4.8 g).



Example 38

Compound 38: To a solution of compound 14 (100 mg, 0.16 mmol) and compound 37 (232 mg, 0.74 mmol) in  $\text{CH}_2\text{Cl}_2$  (1 mL) at  $-40^\circ\text{C}$  was added triflic anhydride (40  $\mu\text{L}$ , 0.24 mmol) slowly. The mixture was warmed to  $25^\circ\text{C}$  slowly, and was stirred for 12 hours. The mixture was concentrated, and was diluted with EtOH/EtOAc (2 mL/0.4 mL). To the above solution at  $0^\circ\text{C}$  was added sodium borohydride (91 mg) in portions. The mixture was stirred at  $0^\circ\text{C}$  for 3 hours, and was diluted with EtOAc. The mixture was washed with saturated sodium bicarbonate, water, and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatograph ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$  to  $100/10$ ) gave the intermediate (33 mg). To the solution of above intermediate in acetonitrile (2.5 mL) was added 48% HF/  $\text{CH}_3\text{CN}$  (0.1 mL). The mixture was stirred for 30 minutes, and was diluted with EtOAc. The organic solution was washed with 0.5 N sodium hydroxide, water, and brine, was dried over  $\text{MgSO}_4$ . Purification by reverse HPLC gave compound 38 (12 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (2 H, d,  $J = 8.9$  Hz), 7.02 (2 H, d,  $J = 8.9$  Hz), 5.70 (1 H, m), 5.45 (1 H, m), 5.05 (1 H, m), 4.2-3.4 (19 H, m), 3.4-2.8 (5 H, m), 2.45-2.20 (4 H, m), 2.15-1.81 (5 H, m), 1.33 (6 H, m), 0.89 (6 H, m).

Scheme 8



I. a.  $\text{SOCl}_2$ /toluene/60 °C; b. ArOH/pyridine; II. a. NaOH/THF/ $\text{H}_2\text{O}$ ; b. HCl;  
 III. b.  $\text{SOCl}_2$ /toluene/60 °C; c. ethyl lactate/pyridine; IV.  $\text{H}_2$ /10% Pd-C/EtOAc/HOAc;  
 V. a. compound 6/ $\text{MgSO}_4$ ; b. HOAc/ $\text{NaCNBH}_3$

Example 39

Compound 39 was prepared by the methods of the previous Examples.

Example 40

Compound 40: To the suspension of compound 39 (4.25 g, 16.4 mmol) in toluene (60 mL) was added thionyl chloride (7.2 mL, 99 mmol), followed by DMF (a few drops). The reaction mixture was heated at 65°C for 5 hrs, and evaporated under reduced pressure. The mixture was coevaporated with toluene (2x) to afford a brown solid. To the solution of the brown solid in  $\text{CH}_2\text{Cl}_2$  (60 mL) at 0°C was added 2,6-dimethylphenol (8.1 g, 66 mmol),

followed by slow addition of pyridine (8 mL, 99 mmol). The reaction mixture was allowed to warm to 25°C and stirred for 14 hrs. Solvents were removed under reduced pressure. The mixture was diluted with EtOAc, and washed with water (3x) and brine (1x), and dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 3/1 to 1/1) afforded compound 40 (1.38 g).

#### Example 41

Compound 41: To a solution of compound 40 (1.38 g, 1.96 mmol) in THF (6 mL) was added 3.55 mL of 1.0 N NaOH solution. The mixture was stirred at 25°C for 24 hours, and THF was removed under reduced pressure. The mixture was diluted with water, and was washed with EtOAc (3x). The aqueous phase was cooled to 0°C, and was acidified with concentrated HCl until pH = 1. The aqueous was extracted with EtOAc (3x). The combined organic layer was washed with water (1x) and brine (1x), and dried over MgSO<sub>4</sub>. Concentration under reduced pressure gave compound 41 as a white solid (860 mg).

#### Example 42

Compound 42: To a suspension of compound 41 (1.00 g, 2.75 mmol) in toluene (15 mL) was added thionyl chloride (1.20 mL, 16.5 mmol), followed by DMF (3 drops). The mixture was heated at 65°C for 5 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) to give a brown solid. To the solution of the above solid in CH<sub>2</sub>Cl<sub>2</sub> (11 mL) at 0°C was added ethyl (s)-lactate (1.25, 11 mmol), followed by pyridine (1.33 mL, 16.6 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was diluted with EtOAc. The organic phase was washed with 1 N HCl, water, and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (hexanes/EtOAc = 1.5/1 to 1/1) gave compound 42 (470 mg).

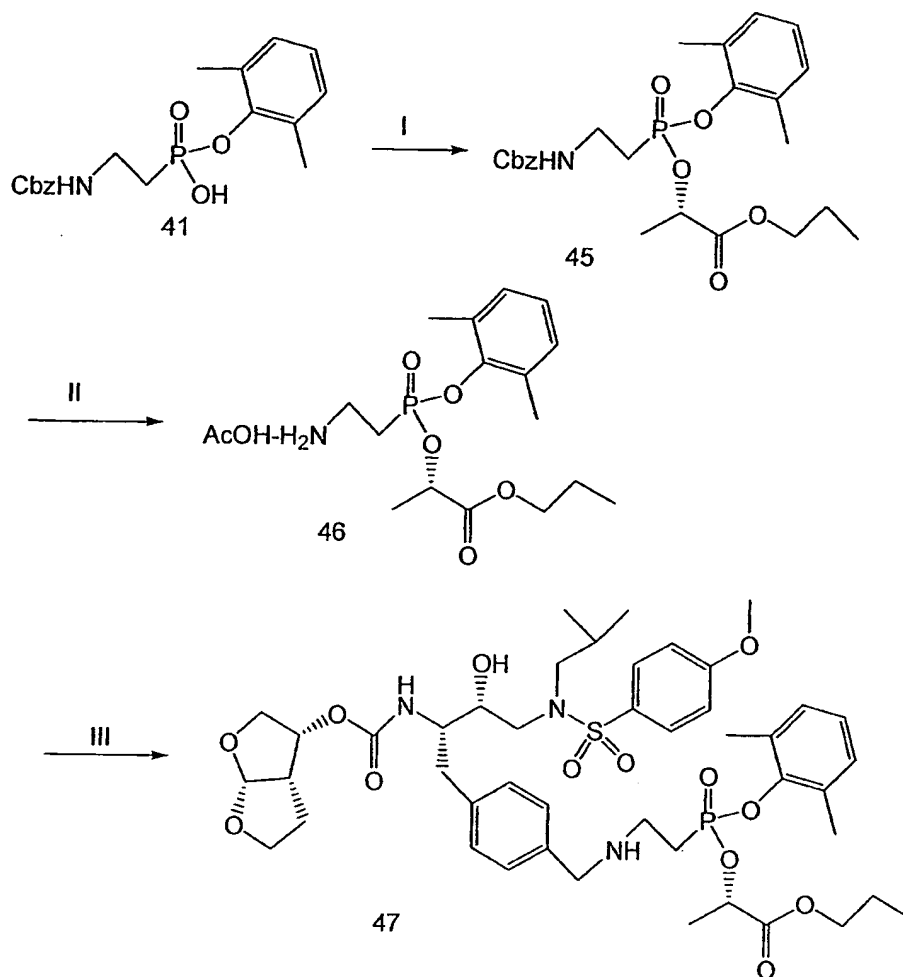
#### Example 43

Compound 43: To a solution of compound 42 (470 mg) in EtOH (10 mL) was added 10% palladium on carbon (90 mg), followed by acetic acid (150 µL). The mixture was hydrogenated for 6 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 43 (400 mg).

Example 44

Compound 44: To a solution of compound 6 (551 mg, 0.93 mmol) in 1,2-dichloroethane (4 mL) was added compound 43 (400 mg, 1.0 mmol), followed by  $\text{MgSO}_4$  (1 g). The mixture was stirred for 3 hours, and acetic acid (148  $\mu\text{L}$ ) and sodium cyanoborohydride (117 mg, 1.86 mmol) were added sequentially. The mixture was stirred for 1 hour. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (EtOAc to EtOAc/EtOH = 9/1) gave compound 44. Compound 44 was dissolved in  $\text{CH}_2\text{Cl}_2$  (25 mL), and trifluoroacetic acid (100  $\mu\text{L}$ ) was added. The mixture was concentrated to give compound 44 as a TFA salt (560 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.74 (2 H, m), 7.39 (2 H, m), 7.20 (2 H, m), 7.03 (5 H, m), 5.68 (1 H, m), 5.43 (1 H, m), 5.01 (1 H, m), 4.79 (1 H, m), 4.35-4.20 (4 H, m), 4.18-3.4 (11 H, m), 3.2-2.6 (9 H, m), 2.30 (6 H, m), 1.82 (1 H, m), 1.70 (2 H, m), 1.40-1.18 (6 H, m), 0.91 (6 H, m).

## Scheme 9



I. b. SOCl<sub>2</sub>/toluene/60°C; c. propyl (s)-lactate/pyridine;  
 II. H<sub>2</sub>/10% Pd-C/EtOAc/HOAc;  
 III. a. compound 6/MgSO<sub>4</sub>; b. HOAc/NaCNBH<sub>3</sub>

## Example 45

Compound 45: To a suspension of compound 41 (863 mg, 2.4 mmol) in toluene (13 mL) was added thionyl chloride (1.0 mL, 14.3 mmol), followed by DMF (3 drops). The mixture was heated at 65°C for 5 hours. The solvent and reagent were removed under reduced pressure. The mixture was coevaporated with toluene (2x) to give a brown solid. To the solution of the above solid in CH<sub>2</sub>Cl<sub>2</sub> (10 mL) at 0°C was added propyl (s)-lactate (1.2 mL, 9.6 mmol), followed by triethylamine (2.0 mL, 14.4 mmol). The mixture was warmed to 25°C and stirred for 12 hours. The reaction mixture was concentrated under reduced pressure, and was

diluted with EtOAc. The organic phase was washed with water and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 1.5/1 to 1/1) gave compound 45 (800 mg).

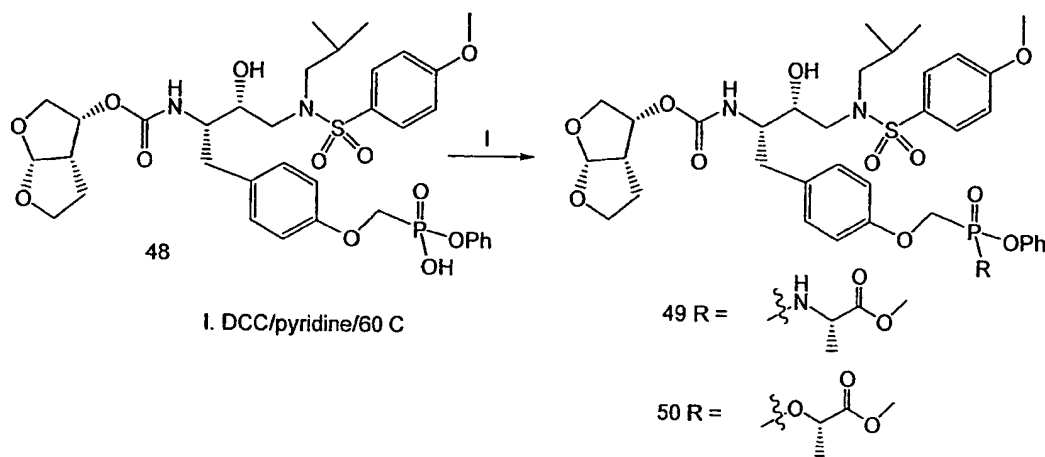
Example 46

Compound 46: To a solution of compound 45 (785 mg) in EtOH (17 mL) was added 10% palladium on carbon (150 mg), followed by acetic acid (250  $\mu\text{L}$ ). The mixture was hydrogenated for 16 hours. The mixture was stirred with celite for 5 mins, and was filtered through a pad of celite. Concentration under reduced pressure gave compound 46 (700 mg).

Example 47

Compound 47: To a solution of compound 6 (550 mg, 0.93 mmol) in 1,2-dichloroethane (4 mL) was added compound 43 (404 mg, 1.0 mmol), followed by  $\text{MgSO}_4$  (1 g). The mixture was stirred for 3 hours, and acetic acid (148  $\mu\text{L}$ ) and sodium cyanoborohydride (117 mg, 1.86 mmol) were added sequentially. The mixture was stirred for 1 hour. The mixture was diluted with EtOAc, and was washed with saturated sodium bicarbonate solution, water (3x) and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (EtOAc to EtOAc/EtOH = 9/1) gave compound 47. Compound 47 was dissolved in  $\text{CH}_2\text{Cl}_2$  (25 mL), and trifluoroacetic acid (100  $\mu\text{L}$ ) was added. The mixture was concentrated to give compound 47 as a TFA salt (650 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.74 (2 H, m), 7.41 (2 H, m), 7.25-7.1 (2 H, m), 7.02 (5 H, m), 5.65 (1 H, m), 5.50 (1 H, m), 5.0-4.75 (2 H, m), 4.25-4.05 (4 H, m), 4.0-3.4 (11 H, m), 3.2-2.6 (9 H, m), 2.31 (6 H, m), 1.82-1.51 (3 H, m), 1.45-1.2 (5 H, m), 0.93 (9 H, m).

Scheme 10

Example 48

Compound 48 was made by the methods of the previous Examples.

Example 49

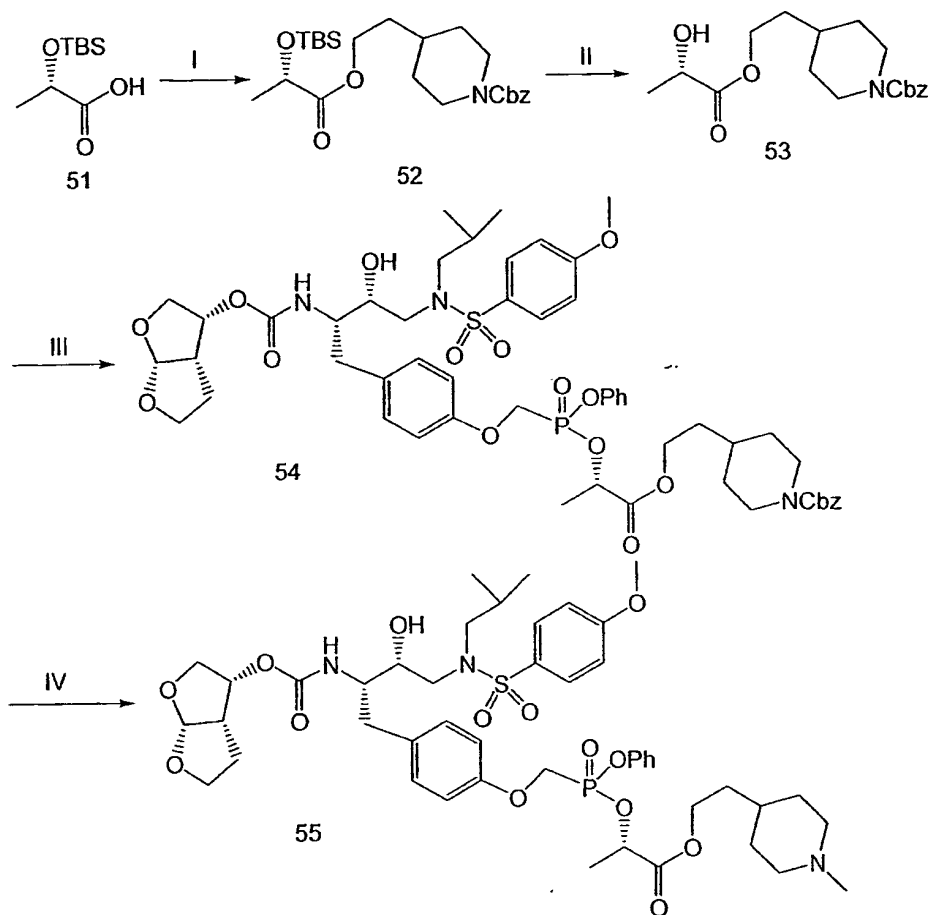
Compound 49: To a solution of compound 48 (100 mg, 0.13 mmol) in pyridine (0.75 mL) was added L-alanine methyl ester hydrochloride (73 mg, 0.52 mmol), followed by DCC (161 mg, 0.78 mmol). The mixture was heated at 60°C for 1 hour. The mixture was diluted with EtOAc, and was washed with 0.2 N HCl, water, 5% sodium bicarbonate, and brine, and was dried over MgSO<sub>4</sub>. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/5) gave compound 49 (46 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.73 (2 H, m), 7.38-7.18 (7 H, m), 7.03 (2 H, m), 6.89 (2 H, m), 5.68 (1 H, m), 5.05 (1 H, m), 4.95 (1 H, m), 4.30 (3 H, m), 4.0-3.6 (12 H, m), 3.2-2.8 (7 H, m), 1.84-1.60 (3 H, m), 1.38 (3 H, m), 0.93 (6 H, m).

Example 50

Compound 50: To a solution of compound 48 (100 mg, 0.13 mmol) in pyridine (0.75 mL) was added methyl (S)-lactate (41 mg, 0.39 mmol), followed by DCC (81 mg, 0.39 mmol). The mixture was heated at 60°C for 2 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered. Purification by flash column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/iPrOH = 100/5) gave compound 50 (83 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.74 (2 H, m), 7.38-7.14 (7 H, m), 7.02 (2 H, m), 6.93 (2 H, m), 5.67 (1 H,

m), 5.18 (1 H, m), 5.04 (1 H, m), 4.92 (1 H, m), 4.5 (2 H, m), 4.0-3.68 (12 H, m), 3.2-2.75 (7 H, m), 1.82 (1 H, m), 1.75-1.50 (5 H, m), 0.93 (6 H, m).

Scheme 11



I. Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/ $\text{ROH}/i\text{Pr}_2\text{NEt}$ ;  
 II. 15%  $\text{HF}/\text{CH}_3\text{CN}$ ; III. Compound 48/ $\text{DCC}/\text{pyridine}/60^\circ\text{C}$ ; IV. a.  $\text{H}_2/10\%\text{Pd-C}$ ;  
 b.  $\text{NaBH}_3\text{CN}/\text{HCHO}/\text{HOAc}$

### Example 51

Compound 51: To a solution of benzyl (s)-lactate (4.0 g, 20 mmol) in DMF (40 mL) was added imidazole (2.7 g, 20 mmol), followed by tert-butyldimethylsilyl chloride (3.3 g, 22 mmol). The mixture was stirred for 14 hours, and diluted with EtOAc. The organic phase was washed with 1.0 N HCl solution (2x), water (2x), and brine (1x), and dried over  $\text{MgSO}_4$ .



Concentration gave the lactate intermediate (6.0 g). To the solution of the above intermediate in EtOAc (200 mL) was added 10% Palladium on carbon (700 mg). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 minutes, and was filtered through a pad of celite. Concentration gave compound 51 (3.8 g).

#### Example 52

Compound 52: To a solution of compound 51 (1.55 g, 7.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) was added 4-benzyloxycarbonylpiperidineethanol (2.00 g, 7.6 mmol), followed by benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (4.74 g, 9.1 mmol) and diisopropylethylamine (1.58 mL, 9.1 mmol). The mixture was stirred for 14 hours, and dichloromethane was removed. The mixture was diluted with EtOAc, and was washed with brine, and dried with  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 10/1) gave compound 52 (1.50 g).

#### Example 53

Compound 53: To a solution of compound 52 (1.50 g) in  $\text{CH}_3\text{CN}$  was added 58% HF/ $\text{CH}_3\text{CN}$  (5 mL). The mixture was stirred for 30 minutes, and acetonitrile was removed under reduced pressure. The mixture was diluted with EtOAc, and was washed with water and brine, and was dried over  $\text{MgSO}_4$ . Purification by flash column chromatography (hexanes/EtOAc = 1/1) gave compound 53 (1.00 g).

#### Example 54

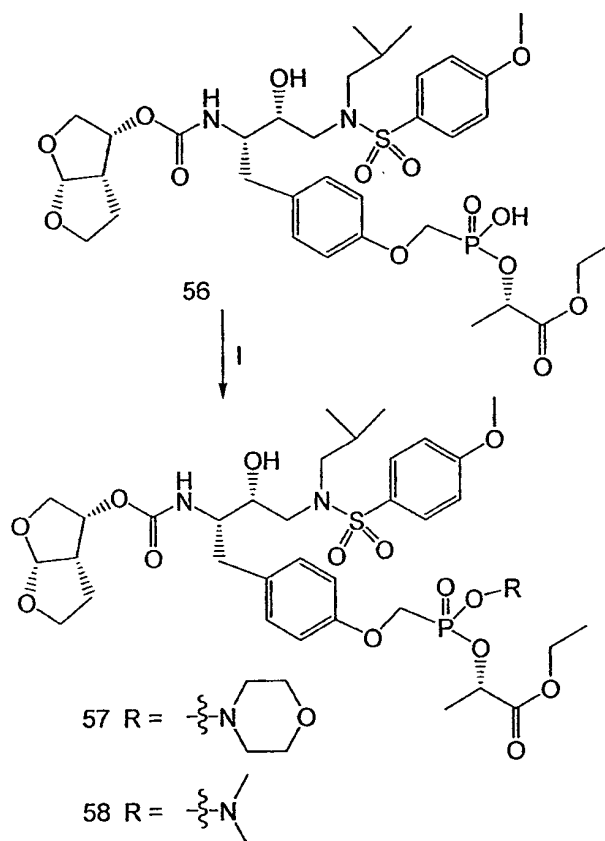
Compound 54: To a solution of compound 48 (769 mg, 1.0 mmol) in pyridine (6.0 mL) was added compound 53 (1.0 g, 3.0 mmol), followed by DCC (618 mg, 3.0 mmol). The mixture was heated at  $60^\circ\text{C}$  for 2 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc (5 mL), and was filtered. Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH}$  = 100/4) gave compound 54 (630 mg).

#### Example 55

Compound 55: To a solution of compound 54 (630 mg, 0.58 mmol) in EtOAc (30 mL) was added 10% Palladium on carbon (63 mg), followed by acetic acid (80  $\mu\text{L}$ ). The mixture was hydrogenated for 2 hours. The mixture was stirred with celite for 5 minutes, and was filtered through a pad of celite. Concentration gave the intermediate. To the solution of the above

intermediate in EtOAc (10 mL) was added 37% formaldehyde (88  $\mu$ L, 1.18 mmol), followed by acetic acid (101  $\mu$ L, 1.77 mmol). The mixture was cooled to 0°C, and sodium cyanoborohydride (74 mg, 1.18 mmol) was added. The mixture was stirred at 25°C for 80 minutes, and was diluted with EtOAc. The mixture was washed with water and brine, and was dried over MgSO<sub>4</sub>. Concentration gave compound 55 as a white solid (530 mg): <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.74 (2 H, m), 7.40-7.15 (7 H, m), 7.03 (2 H, m), 6.92 (2 H, m), 5.66 (1 H, m), 5.20-5.00 (3 H, m), 4.58-4.41 (2 H, m), 4.16 (2 H, m), 4.0-3.7 (9 H, m), 3.4-2.6 (14 H, m), 1.90-1.50 (13 H, m), 0.92 (6 H, m).

### Scheme 12



I. R<sub>2</sub>NOH/DCC/pyridine

### Example 56

Compound 56 was made by the methods of the previous Examples.

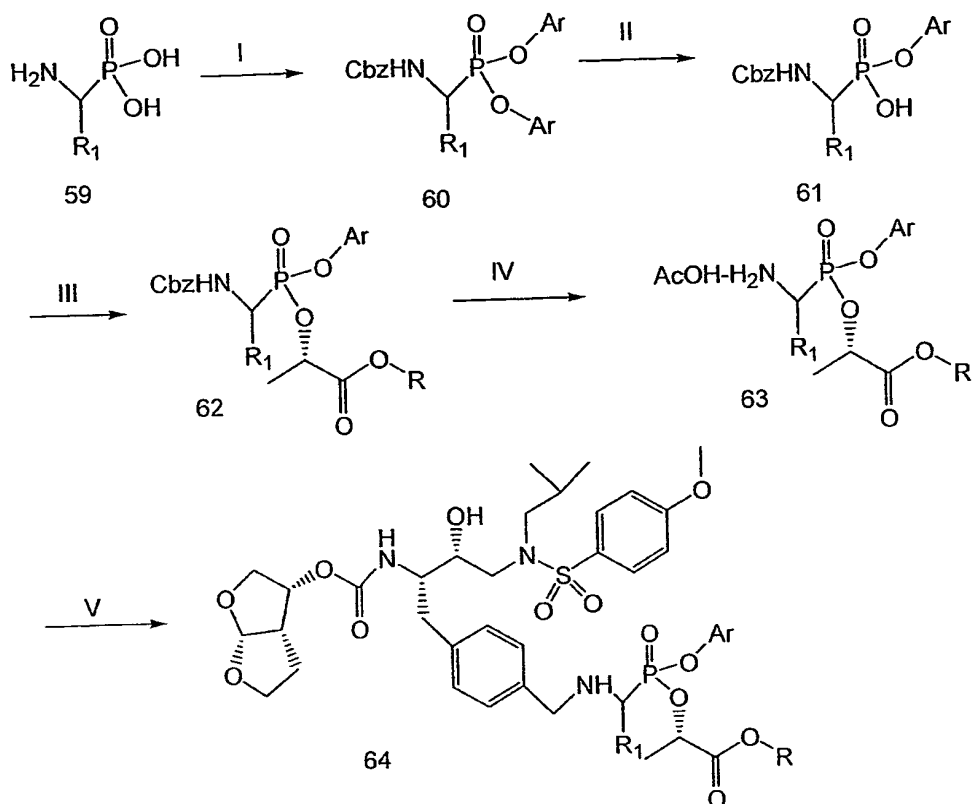
Example 57

Compound 57: To a solution of compound 56 (100 mg, 0.12 mmol) in pyridine (0.6 mL) was added N-hydroxymorpholine (50 mg, 0.48 mmol), followed by DCC (99 mg, 0.48 mmol). The mixture was stirred for 14 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc, and was filtered. Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$ ) gave compound 57 (53 mg):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (2 H, d,  $J = 8.6$  Hz), 7.15 (2 H, d,  $J = 7.6$  Hz), 6.99 (2 H, d,  $J = 8.8$  Hz), 6.90 (2 H, m), 5.67 (1 H, m), 5.18 (1 H, m), 5.05 (1 H, m), 4.95 (1 H, m), 4.58-4.38 (2 H, m), 4.21 (2 H, m), 4.02-3.80 (13 H, m), 3.55-3.38 (2 H, m), 3.2-2.78 (9 H, m), 1.9-1.8 (1 H, m), 1.8-0.95 (5 H, m), 1.29 (3 H, m), 0.93 (6 H, m).

Example 58

Compound 58: To a solution of compound 56 (100 mg, 0.12 mmol) in pyridine (0.6 mL) was added N,N-dimethylhydroxylamine hydrochloride (47 mg, 0.48 mmol), followed by DCC (99 mg, 0.48 mmol). The mixture was stirred for 6 hours, and pyridine was removed under reduced pressure. The mixture was diluted with EtOAc, and was filtered. Purification by flash column chromatography ( $\text{CH}_2\text{Cl}_2/\text{iPrOH} = 100/5$ ) gave compound 58 (35 mg).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (2 H, d,  $J = 8.9$  Hz), 7.15 (2 H, d,  $J = 8.2$  Hz), 6.99 (2 H, d,  $J = 8.4$  Hz), 6.89 (2 H, m), 5.65 (1 H, d,  $J = 5.2$  Hz), 5.15 (1 H, m), 4.98 (2 H, m), 4.42 (2 H, m), 4.18 (2 H, m), 4.0-3.6 (9 H, m), 3.2-2.7 (13 H, m), 1.92-1.45 (6 H, m), 1.25 (3 H, m), 0.90 (6 H, m).

Scheme 13



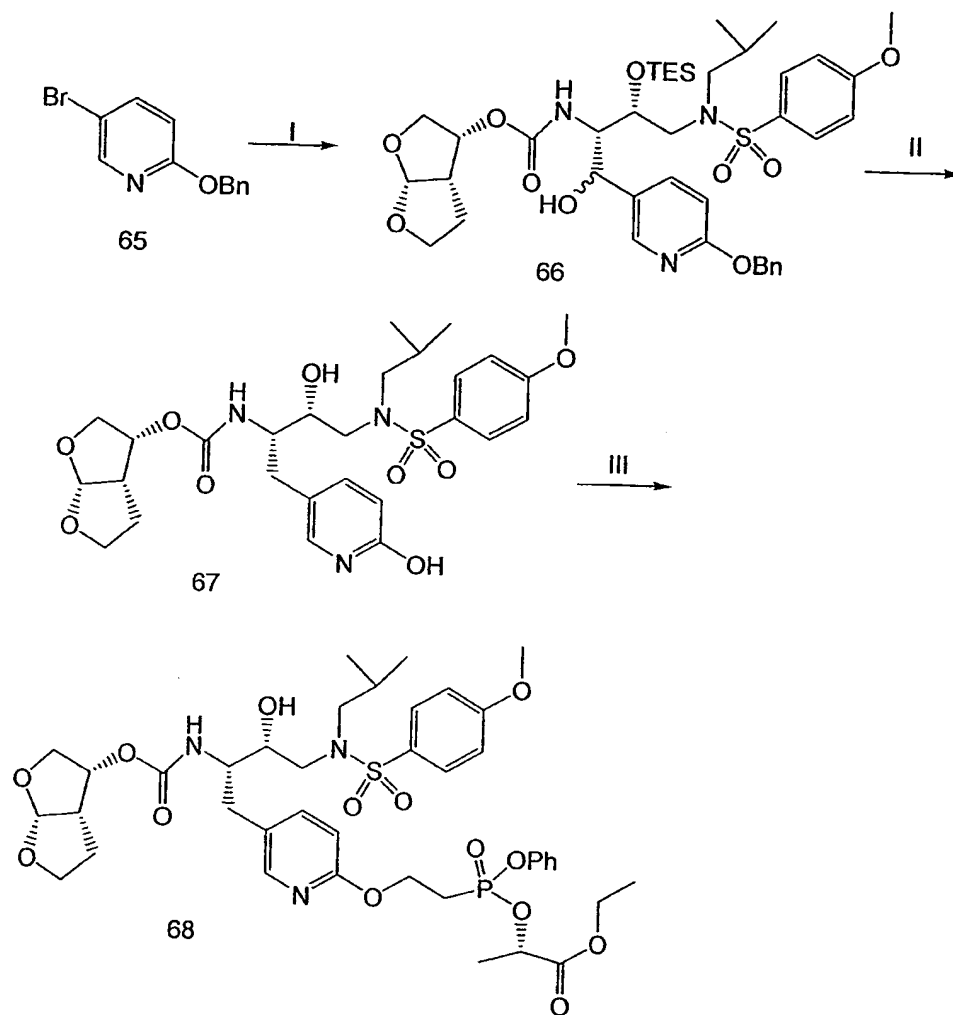
R = Me, Et, Pr, i-Pr; R<sub>1</sub> = H, Me, Et, i-Pr; Ar = phenyl, 2, 6-dimethylphenyl

I. a. CbzCl/NaOH; b. SOCl<sub>2</sub>/toluene/60 °C; c. ArOH/pyridine; II. a. NaOH/THF/H<sub>2</sub>O; b. HCl  
 III. a. SOCl<sub>2</sub>/toluene/60 °C; b. alkyl (s)-lactate/pyridine; IV. H<sub>2</sub>/10% Pd-C/EtOAc/HOAc;  
 V. a. compound 6/MgSO<sub>4</sub>; b. HOAc/NaCNBH<sub>3</sub>

Aminomethylphosphonic acid 59 is protected as benzyl carbamate. The phosphonic acid is treated with thionyl chloride to generate dichloridate, which reacts with phenyl or 2,6-dimethylphenol to give compound 60. Compound 60 is hydrolyzed with sodium hydroxide, followed by acidification to afford monoacid 61. Monoacid 61 is treated with thionyl chloride to generate monochloridate, which reacts with different alkyl (s)-lactates to form compound 62. Compound 62 is hydrogenated with 10% Pd-C in the presence of acetic acid to

give compound 63. Compound 63 reacts with aldehyde 6 in the presence of  $\text{MgSO}_4$  to form imine, which is reduced with sodium cyanoborohydride to generate compound 64.

Scheme 14

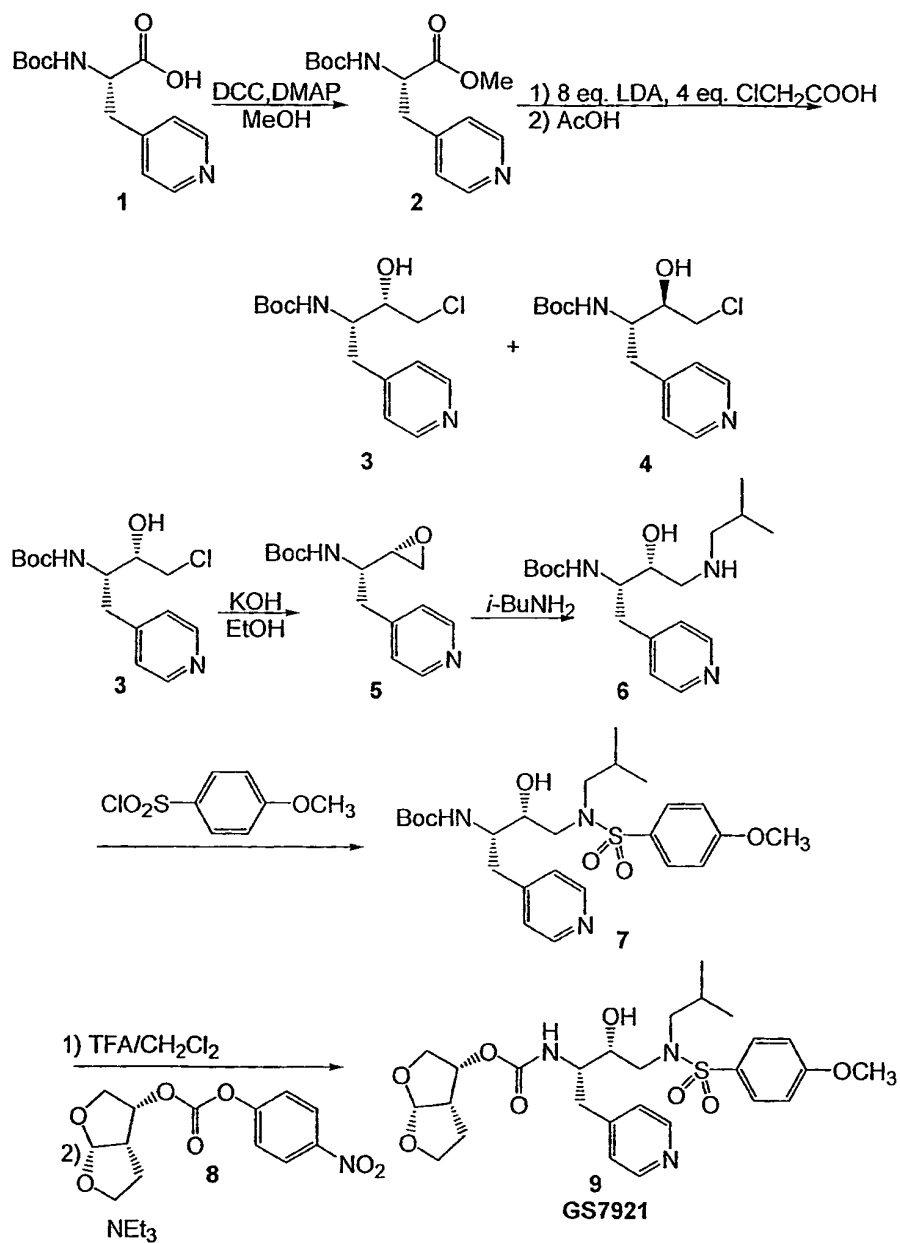


I.a. *n*-BuLi; b. compound 15; II.  $\text{H}_2/10\%\text{Pd-C}/\text{HOAc}$ ; IV.  $\text{PPh}_3/\text{DEAD}$

Compound 65 is prepared from 2-hydroxy-5-bromopyridine by alkylation. J. Med. Chem. 1992, 35, 3525. Compound 65 is treated with *n*-Butyl lithium to generate aryl lithium, which reacts with aldehyde 15 to form compound 66. J. Med. Chem. 1994, 37, 3492. Compound 66 is hydrogenated with 10% Pd-C in the presence of acetic acid to give compound 67. J. Med. Chem. 2000, 43, 721. Compound 68 is prepared from compound 67

with corresponding alcohol under Mitsunobu reaction conditions. Bioorg. Med.Chem. Lett. 1999, 9, 2747.

Scheme 1



Example 1

Methyl 2-(*S*)-(dimethylethoxycarbonylamino)-3-(4-pyridyl)propanoate (2): A solution of *N*-tert-Butoxycarbonyl-4-pyridylalanine (1, 9.854 g, 37 mmol, Peptech), 4-dimethylaminopyridine (4.52 g, 37 mmol, Aldrich), and dicyclohexylcarbodiimide (15.30 g, 74.2 mmol, Aldrich) in methanol (300 mL) was stirred at 0°C for 2 h and at room temperature for 12 h. After the solids were removed by filtration, the filtrate was concentrated under reduced pressure. More dicyclohexylurea was removed by repeated trituration of the concentrated residue in EtOAc followed by filtration. The residue was chromatographed on silica gel to afford the methyl ester 2 (9.088 g, 88%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.53 (d, 2H, *J* = 5.7 Hz), 7.09 (d, 2H, *J* = 5.7 Hz), 5.04 (br, 1H), 4.64 (br, 1H), 3.74 (s, 3H), 3.16 (dd, 1H, *J* = 13.5 and 5.7 Hz), 3.02 (dd, 1H, *J* = 13.5 and 6.3 Hz), 1.42 (s, 9H); MS (ESI) 281 (M+H).

Example 2

1-Chloro-3-(*S*)-(dimethylethoxycarbonylamino)-4-(4-pyridyl)-2-(*S*)-butanol (3): A solution of diisopropylamine (37.3 mL, 266 mmol, Aldrich) in THF (135 mL) was stirred at -78°C as a solution of *n*-butyllithium (102 mL of 2.3 M solution and 18 mL of 1.4 M solution 260 mmol, Aldrich) in hexane was added. After 10 min, the cold bath was removed and stirred the solution for 10 min at the ambient temperature. The solution was cooled at -78°C again and stirred as a solution of chloroacetic acid (12.255 g, 130 mmol, Aldrich) in THF (50 mL) was added over 20 min. After the solution was stirred for 15 min, this dianion solution was transferred to a stirred solution of the methyl ester 2 (9.087 g, 32.4 mmol) in THF (100 mL) at 0°C over 15 min. The resulting yellow slurry was stirred at 0°C for 10 min and cooled at -78°C. A solution of acetic acid (29 mL, 507 mmol, Aldrich) in THF (29 mL) was added quickly to the slurry and the resulting slurry was stirred at -78°C for 30 min, at 0°C for 30 min, and at room temperature for 15 min. The resulting slurry was dissolved in saturated NaHCO<sub>3</sub> solution (750 mL) and EtOAc (500 mL). The separated aqueous layer was extracted with EtOAc (300 mL x 2) and the combined organic fractions were washed with water (750 mL x 2) and saturated NaCl solution (250 mL). The resulting solution was dried (MgSO<sub>4</sub>) and evaporated under reduced pressure.

A solution of the residue in THF (170 mL) and water (19 mL) was stirred at 0°C as NaBH<sub>4</sub> (3.375 g, 89.2 mmol, Aldrich) was added. After 30 min, the solution was evaporated under reduced pressure and the residue was dissolved in EtOAc, acidified with aqueous NaHSO<sub>4</sub>,

and then neutralized by adding saturated aqueous  $\text{NaHCO}_3$  solution. The separated aqueous fraction was extracted with EtOAc (100 mL) and the combined organic fractions were washed with water (500 mL) and saturated NaCl solution (100 mL). The solution was dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The residue was chromatographed on silica gel to afford the chlorohydrin 3 and 4 (4.587 g, 47%) as a mixture of two diastereomers (3~4:1). The obtained mixture was recrystallized from EtOAc-hexane twice to obtain pure desired diastereomer 3 (2.444 g, 25%) as yellow crystals:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.53 (d, 2H,  $J = 5.7$  Hz), 7.18 (d, 2H,  $J = 5.7$  Hz), 4.58 (br, 1H), 3.94 (m, 1H), 3.87 (br, 1H), 3.75-3.54 (m, 2H), 3.05 (dd, 1H,  $J = 13.8$  and 3.9 Hz), 2.90 (dd, 1H,  $J = 13.8$  and 8.4 Hz), 1.36 (s, 9H); MS (ESI) 301 (M+H).

#### Example 3

The epoxide 5: A solution of the chlorohydrin 3 (1.171 g, 3.89 mmol) in ethanol (39 mL) was stirred at room temperature as 0.71 M KOH in ethanol (6.6 mL) was added. After 1.5 h, the mixture was concentrated under reduced pressure and the residue was dissolved in EtOAc (60 mL) and water (60 mL). The separated aqueous fraction was extracted with EtOAc (60 mL) and the combined organic fractions were washed with saturated NaCl solution, dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure to obtain the epoxide (1.058 g, quantitative):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.52 (d, 2H,  $J = 6.0$  Hz), 7.16 (d, 2H,  $J = 6.0$  Hz), 4.57 (d, 1H,  $J = 7.8$  Hz), 3.76 (br, 1H), 3.02-2.92 (m, 2H), 2.85-2.79 (m, 2H), 2.78-2.73 (m, 1H), 1.37 (s, 9H); MS (ESI) 265 (M+H).

#### Example 4

The hydroxy-amine 6: A solution of the epoxide 5 obtained above and *i*-BuNH<sub>2</sub> (3.9 mL, 39.2 mmol, Aldrich) in 58 mL of *i*-PrOH was stirred at 65°C for 2 h and the solution was concentrated under reduced pressure. The residual *i*-PrOH was removed by dissolving the residue in toluene and concentration of the solution twice:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.51 (d, 2H,  $J = 6.0$  Hz), 7.18 (d, 2H,  $J = 6.0$  Hz), 4.70 (d, 1H,  $J = 9.6$  Hz), 3.86 (br, 1H), 3.46 (q, 1H,  $J = 5.8$  Hz), 3.06 (dd, 1H,  $J = 14.1$  and 3.9 Hz), 2.79 (dd, 1H,  $J = 14.1$  and 9.0 Hz), 2.76-2.63 (m, 3H), 2.43 (m, 2H,  $J = 6.9$  Hz), 1.73 (m, 1H,  $J = 6.6$  Hz), 1.36 (s, 9H), 0.93 (d, 3H,  $J = 6.6$  Hz), 0.92 (d, 3H,  $J = 6.6$  Hz); MS (ESI) 338 (M+H).

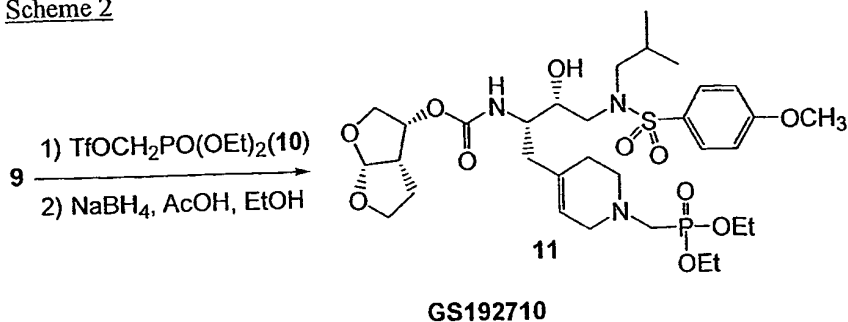


Example 5

The sulfoamide 7: A solution of the crude 6 and *p*-methoxybenzene sulfonyl chloride (890 mg, 4.31 mmol, Aldrich) in CH<sub>2</sub>Cl<sub>2</sub> (24 mL) was stirred at 0°C for 2 h and at room temperature for 13 h. The solution was washed with saturated NaHCO<sub>3</sub> solution and the aqueous washing was extracted with CH<sub>2</sub>Cl<sub>2</sub> (60 mL). After the combined organic fractions were dried (MgSO<sub>4</sub>) and concentrated under reduced pressure, the residue was purified by chromatography on silica gel to obtain the sulfoamide 7 (1.484 g, 75%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.51 (d, 2H, *J* = 5.7 Hz), 7.73 (d, 2H, *J* = 8.7 Hz), 7.21 (d, 2H, *J* = 5.7 Hz), 7.00 (d, 2H, *J* = 8.7 Hz), 4.68 (d, 1H, *J* = 8.1 Hz), 4.08 (br, 1H), 3.88 (s, 3H), 3.83 (br, 2H), 3.09 (d, 2H, *J* = 5.1 Hz), 3.06-2.80 (m, 4H), 1.85 (m, 1H, *J* = 7.0 Hz), 1.34 (s, 9H), 0.92 (d, 3H, *J* = 6.3 Hz), 0.89 (d, 3H, *J* = 6.6 Hz); MS (ESI) 508 (M+H).

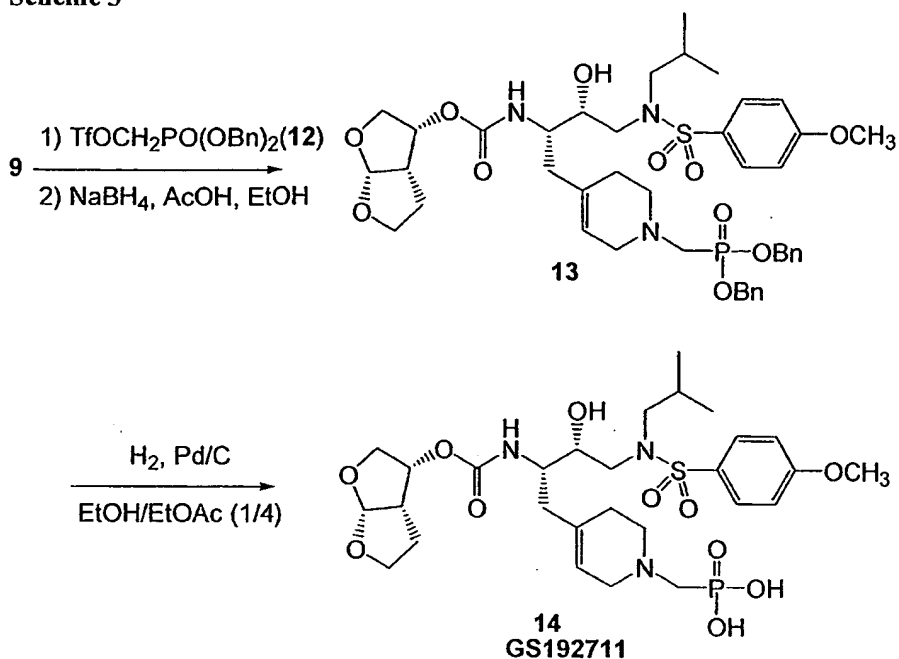
Example 6

The bisfurancarbamate 9: A solution of the sulfoamide 7 (1.484 g, 2.92 mmol) and trifluoroacetic acid (6.8 mL, 88.3 mmol, Aldrich) in CH<sub>2</sub>Cl<sub>2</sub> (18 mL) was stirred at room temperature for 2 h. After the solution was evaporated under reduced pressure, the residue was dissolved in acetonitrile (10 mL) and toluene (10 mL), and evaporated to dryness twice to result crude amine as TFA salt. A solution of the crude amine, dimethylaminopyridine (72 mg, 0.59 mmol, Aldrich), diisopropylethylamine (2.55 mL, 14.6 mmol, Aldrich) in acetonitrile was stirred at 0°C as the bisfurancarboxylate 8 (907 mg, 3.07 mmol, obtained from Azar) was added in portion. The solution was stirred at 0°C for 1 h and at room temperature for 19 h, and concentrated under reduced pressure. The residue was dissolved in EtOAc (60 mL) and washed with saturated NaHCO<sub>3</sub> solution (60 mL). After the aqueous washing was extracted with EtOAc (60 mL), the combined organic fractions were washed with saturated NaHCO<sub>3</sub> (60 mL) and saturated NaCl solution (60 mL), dried (MgSO<sub>4</sub>), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to obtain the carbamate 9 (1.452 g, 88%): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 8.50 (d, 2H, *J* = 5.7 Hz), 7.72 (d, 2H, *J* = 8.7 Hz), 7.19 (d, 2H, *J* = 5.7 Hz), 7.01 (d, 2H, *J* = 8.7 Hz), 5.65 (d, 1H, *J* = 5.1 Hz), 5.12 (d, 1H, *J* = 9.3 Hz), 5.02 (q, 1H, *J* = 6.7 Hz), 4.01-3.77 (m, 4H), 3.88 (s, 3H), 3.76-3.63 (m, 2H), 3.18-2.76 (m, 7H), 1.95-1.77 (m, 1H), 1.77-1.56 (m, 2H), 1.56-1.41 (m, 1H), 0.94 (d, 3H, *J* = 6.6 Hz), 0.90 (d, 3H, *J* = 6.9 Hz); MS (ESI) 564 (M+H).

Scheme 2Example 7

The tetrahydropyridine-diethyl phosphonate **11**: A solution of the pyridine **9** (10.4 mg, 0.018 mmol) and the triflate **10** (8.1 mg, 0.027 mmol, in acetone- $d_6$  (0.75 mL) was stored at room temperature for 9 h and the solution was concentrated under reduced pressure:  $^{31}\text{P}$  NMR (acetone- $d_3$ )  $\delta$  14.7; MS (ESI) 714 ( $\text{M}^+$ ). The concentrated crude pyridinium salt was dissolved in ethanol (2 mL) and stirred at room temperature as  $\text{NaBH}_4$  (~10 mg, Aldrich) was added occasionally over 4 h. To the mixture was added a solution of acetic acid (0.6 mL, Aldrich) in ethanol (3 mL) until the pH of the mixture became 3~4. More  $\text{NaBH}_4$  and acetic acid were added until the reaction was completed. The mixture was carefully concentrated under reduced pressure and the residue was dissolved in saturated  $\text{NaHCO}_3$  solution (10 mL). The product was extracted using  $\text{EtOAc}$  (10 mL x 3) and washed with saturated  $\text{NaCl}$  solution, dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to obtain the product **11** (8.5 mg, 64%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (d, 2H,  $J = 8.7$  Hz), 7.00 (d, 2H,  $J = 8.7$  Hz), 5.71 (d, 1H,  $J = 5.1$  Hz), 5.41 (br, 1H), 5.15-5.08 (m, 1H), 5.00 (br, 1H), 4.14 (dq, 4H,  $J = 7.2$  Hz), 4.06-3.94 (m, 2H), 3.88 (s, 3H), 3.92-3.80 (m, 2H), 3.75 (dd, 1H,  $J = 9.6$  and 6.6 Hz), 3.79-3.61 (m, 1H), 3.24-2.94 (m, 6H), 2.85 (d, 2H,  $J = 11.7$  Hz), 2.88-2.76 (m, 2H), 2.75-2.63 (m, 1H), 2.38-2.29 (m, 1H), 2.24-2.2.12 (m, 2H), 2.12-1.78 (m, 4H), 1.30 (t, 6H,  $J = 7.1$  Hz), 0.94 (d, 3H,  $J = 6.6$  Hz), 0.91 (d, 3H,  $J = 6.3$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  24.6; MS (ESI) 740 ( $\text{M}+\text{Na}$ ).

## Scheme 3



## Example 8

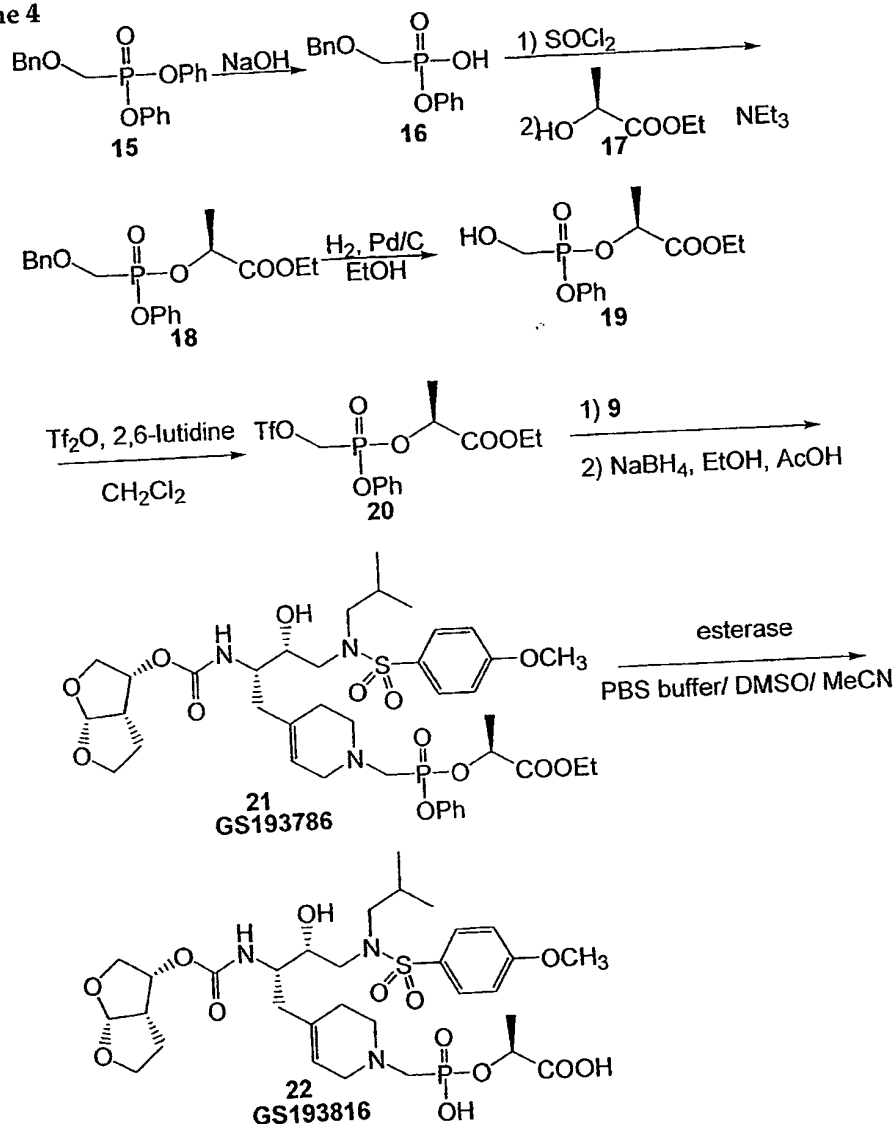
The tetrahydropyridine-dibenzyl phosphonate 13: The compound 13 was obtained by the same procedure as described for compound 11 using the pyridine 9 (10.0 mg, 0.018 mmol) and the triflate 12 (9.4 mg, 0.022 mmol). The product 13 was purified by preparative TLC to afford the dibenzyl phosphonate 13 (8.8 mg, 59%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (d, 2H,  $J = 8.7$  Hz), 7.35 (s, 10H), 7.00 (d, 2H,  $J = 8.7$  Hz), 5.65 (d, 1H,  $J = 5.1$  Hz), 5.39 (br, 1H), 5.15-4.92 (m, 6H), 4.03-3.77 (m, 6H), 3.77-3.62 (m, 2H), 3.56 (br, 1H), 3.24-2.62 (m, 9H), 2.32 (d, 1H,  $J = 13.5$  Hz), 2.24-1.75 (m, 6H), 0.94 (d, 3H,  $J = 6.6$  Hz), 0.89 (d, 3H,  $J = 6.3$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  25.5; MS (ESI) 842 ( $\text{M}+\text{H}$ ).

## Example 9

The phosphonic acid 14: A mixture of the dibenzyl phosphonate 13 (8.8 mg, 0.011 mmol) and 10%  $\text{Pd/C}$  in  $\text{EtOAc}$  (2 mL) and  $\text{EtOH}$  (0.5 mL) was stirred under  $\text{H}_2$  atmosphere for 10 h at room temperature. After the mixture was filtered through celite, the filtrate was concentrated to dryness to afford the product 14 (6.7 mg, quantitative):  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.76 (d, 2H,  $J = 9.0$  Hz), 7.10 (d, 2H,  $J = 9.0$  Hz), 5.68 (d, 1H,  $J = 5.1$  Hz), 5.49 (br, 1H), 5.11 (m, 1H), 3.90 (s, 3H), 4.04-3.38 (m, 10H), 3.22 (d, 2H,  $J = 12.9$  Hz), 3.18-3.00 (m, 2H),

2.89-2.75 (m, 2H), 2.68-2.30 (m, 3H), 2.21-1.80 (m, 4H), 0.92 (d, 3H,  $J = 6.3$  Hz), 0.85 (d, 3H,  $J = 6.3$  Hz);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  6.29; MS (ESI) 662 ( $\text{M}+\text{H}$ ).

Scheme 4

**Example 10**

Diphenyl benzyloxymethylphosphonate 15: To a solution of diphenylphosphite (46.8 g, 200 mmol, Aldrich) in acetonitrile (400 mL) (at ambient temperature) was added potassium carbonate (55.2 g, 400 mmol) followed by the slow addition of benzyl chloromethyl ether (42

mL, 300 mmol, about 60%, Fluka). The mixture was stirred overnight, and was concentrated under reduced pressure. The residue was dissolved in EtOAc, washed with water, saturated NaCl, dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated. The crude product was chromatographed on silica gel to afford the benzylether (6.8 g, 9.6%) as a colorless liquid.

#### Example 11

Monoacid 16: To a solution of diphenyl benzyloxymethylphosphonate 15 (6.8 g, 19.1 mmol) in THF (100 mL) at room temperature was added 1N NaOH in water (21 mL, 21 mmol). The solution was stirred 3 h. The THF was evaporated under reduced pressure and water (100 mL) was added. The aqueous solution was cooled to 0°C, neutralized to pH 7 with 3N HCl and washed with EtOAc. The aqueous solution was again cooled to 0°C, acidified with 3N HCl to pH 1, saturated with sodium chloride, and extracted with EtOAc. The organic layer was washed with brine and dried ( $\text{Na}_2\text{SO}_4$ ), filtered and evaporated, then co-evaporated with toluene to yield the monoacid (4.0 g, 75%) as a colorless liquid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.28-7.09 (m, 10H), 4.61 (s, 2H), 3.81 (d, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.8.

#### Example 12

Ethyl lactate phosphonate 18: To a solution of monoacid 16 (2.18 g, 7.86 mmol) in anhydrous acetonitrile (50 mL) under a nitrogen atmosphere was slowly added thionyl chloride (5.7 mL, 78 mmol). The solution was stirred in a 70°C oil bath for three hours, cooled to room temperature and concentrated. The residue was dissolved in anhydrous dichloromethane (50 mL), and this solution cooled to 0°C and stirred under a nitrogen atmosphere. To the stirring solution was added ethyl (S)-(-)-lactate (2.66 mL, 23.5 mmol) and triethylamine (4.28 mL, 31.4 mmol). The solution was warmed to room temperature and allowed to stir for one hour. The solution was diluted with ethyl acetate, washed with water, brine, citric acid and brine again, dried ( $\text{MgSO}_4$ ), filtered through Celite, concentrated under reduced pressure and chromatographed on silica gel using 30% ethylacetate in hexane. The two diastereomers were pooled together.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.40-7.16 (m, 20H), 5.18-5.13 (m, 2H), 4.73 (s, 2H), 4.66 (d, 2H), 4.28-4.11 (m, 5H), 4.05 (d, 2H), 3.95 (d, 2H), 1.62 (d, 3H), 1.46 (d, 3H), 1.30-1.18 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.6, 17.7.

Example 13

Ethyl lactate phosphonate with free alcohol 19: Ethyl lactate phosphonate 18 was dissolved in EtOH (50mL) and under a nitrogen atmosphere 10% Pd-C (approximately 20 wt %) was added. The nitrogen atmosphere was replaced with hydrogen (1atm) and the suspension stirred for two hours. 10% Pd-C was again added (20 wt %) and the suspension stirred five hours longer. Celite was added, the reaction mixture was filtered through Celite and the filtrate was concentrated to afford 1.61 g (71% from monoacid 16) of the alcohol as a colorless liquid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.40-7.16 (m, 10H), 5.16-5.03 (m, 2H), 4.36-4.00 (m, 8H), 1.62 (d, 3H), 1.46 (d, 3H), 1.30-1.22 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.3, 20.0.

Example 14

Triflate 20: To a solution of ethyl lactate phosphonate with free alcohol 19 (800 mg, 2.79 mmol) in anhydrous dichloromethane (45 mL) chilled to  $-40^\circ\text{C}$  under a nitrogen atmosphere was added triflic anhydride (0.516 mL, 3.07 mmol) and 2-6 lutidine (0.390 mL, 3.34 mmol). The solution was stirred for 3 hr, then warmed to  $-20^\circ\text{C}$  and stirred one hour longer. 0.1 equivalents of triflic anhydride and 2-6 lutidine were then added and stirring was resumed for 90 minutes more. The reaction mixture was diluted with ice-cold dichloromethane, washed with ice-cold water, washed with ice-cold brine and the organic layer was dried ( $\text{MgSO}_4$ ) and filtered. The filtrate was concentrated and chromatographed on silica gel using 30% EtOAc in hexane as eluent to afford 602 mg (51%) of the triflate diastereomers as a slightly pink, transparent liquid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.45-7.31 (m, 4H), 7.31-7.19 (m, 6H), 5.15-4.75 (m, 6H), 4.32-4.10 (4H), 1.62 (d, 3H), 1.50 (d, 3H), 1.30-1.22 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.3, 8.3.

Example 15

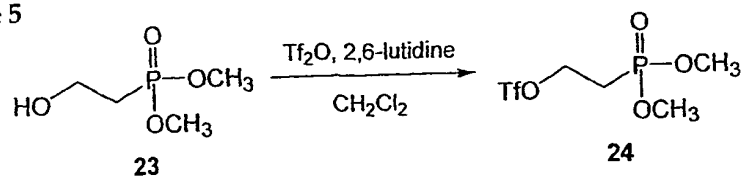
The tetrahydropyridine-prodrug 21: A solution of the pyridine 9 (11.1 mg, 0.020 mmol) and the triflate 20 (11.4 mg, 0.027 mmol) in acetone- $\text{d}_6$  (0.67 mL, Aldrich) was stored at room temperature for 7 h and the solution was concentrated under reduced pressure:  $^{31}\text{P}$  NMR (acetone- $\text{d}_6$ )  $\delta$  11.7, 10.9; MS (ESI) 838 (M+H). The concentrated crude pyridinium salt was dissolved in ethanol (1 mL) and added 2~3 drops of a solution of acetic acid (0.6 mL, Aldrich) in ethanol (3 mL). The solution was stirred at  $0^\circ\text{C}$  as  $\text{NaBH}_4$  (7~8 mg, Aldrich) was

added. More acetic acid solution was added to adjust pH 3-4 of the reaction mixture. Additions of NaBH<sub>4</sub> and the acetic acid solution were repeated until the reaction was completed. The mixture was carefully concentrated under reduced pressure and the residue was purified by chromatography on C18 reverse phase column material followed by preparative TLC using C18 reverse phase plate to obtain the prodrug **21** (13.6 mg, 70%) as a 2:3 mixture of two diastereomers: <sup>1</sup>H NMR (CD<sub>3</sub>CN) δ 7.78 (d, 2H, *J* = 9.0 Hz), 7.48-7.42 (m, 2H), 7.35-7.27 (m, 3H), 7.10 (d, 2H, *J* = 9.0 Hz), 5.86 (m, 1H), 5.60 (m, 1H), 5.48 (br, 1H), 5.14-5.03 (m, 2H), 4.29-4.13 (m, 2H), 3.89 (s, 3H), 3.97-3.32 (m, 12H), 3.29 (br, 0.4H), 3.24 (br, 0.6H), 3.02-2.82 (m, 4H), 2.64-2.26 (m, 3H), 2.26-2.08 (m, 1H), 1.94-1.76 (m, 3H), 1.57 (d, 1.8H, *J* = 6.9 Hz), 1.46 (d, 1.2H, *J* = 6.9 Hz), 1.28 (d, 1.2H, *J* = 6.9 Hz), 1.21 (d, 1.8H, *J* = 7.2 Hz), 0.92-0.88 (m, 6H); <sup>31</sup>P NMR (CD<sub>3</sub>CN) δ 14.4 (0.4P), 13.7 (0.6P); MS (ESI) 838 (M+H).

#### Example 16

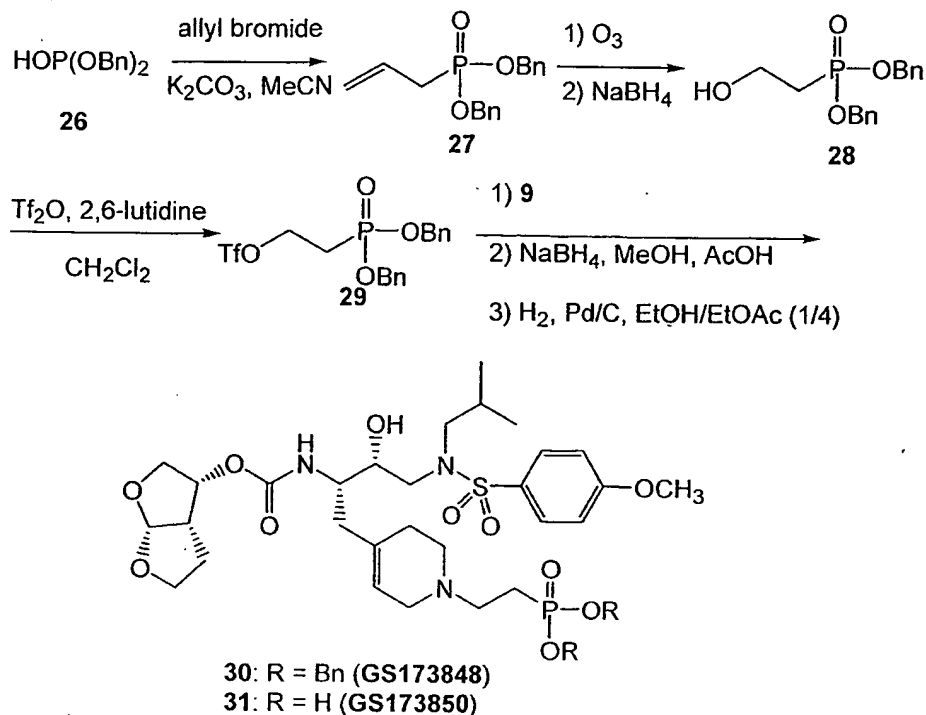
Metabolite 22: To a solution of the prodrug **21** (10.3 mg, 0.011 mmol) in DMSO (0.1 mL) and acetonitrile (0.2 mL) was added 0.1 M PBS buffer (3 mL) mixed thoroughly to result a suspension. To the suspension was added porcine liver esterase suspension (0.05 mL, EC3.1.1.1, Sigma). After the suspension was stored in 37°C for 1.5 h, the mixture was centrifuged and the supernatant was taken. The product was purified by HPLC and the collected fraction was lyophilized to result the product **22** as trifluoroacetic acid salt (7.9 mg, 86%): <sup>1</sup>H NMR (D<sub>2</sub>O) δ 7.70 (d, 1H), 7.05 (d, 2H), 5.66 (d, 1H), 5.40 (br, 1H), 5.02 (br, 1H), 4.70 (br, 1H), 3.99-3.89 (m, 2H), 3.81 (s, 3H), 3.83-3.50 (m, 8H), 3.34-2.80 (m, 7H), 2.50-2.18 (m, 3H), 2.03 (m, 1H), 1.92-1.70 (m, 3H), 1.39 (d, 3H), 0.94 (d, 3H), 0.93 (d, 3H); <sup>31</sup>P NMR (D<sub>2</sub>O) δ 9.0, 8.8; MS (ESI) 734 (M+H).

Scheme 5





Scheme 6



#### Example 19

Dibenzyl phosphonate with double bond 27: To a stirring solution of allyl bromide (4.15 g, 34 mmol, Aldrich) and dibenzylphosphite (6 g, 23 mmol, Aldrich) in acetonitrile (25 mL) was added potassium carbonate (6.3 g, 46 mmol, powder 325 mesh Aldrich) to create a suspension, which was heated to 65°C and stirred for 72 hours. The suspension was cooled to room temperature, diluted with ethyl acetate, filtered, and the filtrate was washed with water, then brine, dried (MgSO<sub>4</sub>), concentrated and used directly in the next step.

#### Example 20

Dibenzylhydroxyethylphosphonate 28: Dibenzyl phosphonate with double bond 27 was dissolved in methanol (50mL), chilled to -78°C, stirred, and subjected to ozone by bubbling ozone into the solution for three hours until the solution turned pale blue. The ozone flow was stopped and oxygen bubbling was done for 15 minutes until the solution became colorless. Sodium borohydride (5 g, excess) was added slowly portionwise. After the evolution of gas subsided the solution was allowed to warm to room temperature, concentrated, diluted with ethyl acetate, made acidic with acetic acid and water and

partitioned. The ethyl acetate layer was washed with water, then brine and dried ( $\text{MgSO}_4$ ), filtered, concentrated and chromatographed on silica gel eluting with a gradient of eluent from 50% ethyl acetate in hexane to 100% ethyl acetate, affording 2.76 g of the desired product.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.36 (m, 10H), 5.16-4.95 (m, 4H), 3.94-3.80 (dt, 2H), 2.13-2.01 (dt, 2H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  31.6.

#### Example 21

Dibenzyl phosphonate 30: A solution of the alcohol 28 (53.3 mg, 0.174 mmol) and 2,6-lutidine (0.025 mL, 0.215 mmol, Aldrich) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was stirred at  $-45^\circ\text{C}$  as trifluoromethanesulfonic anhydride (0.029 mL, 0.172 mmol, Aldrich) was added. The solution was stirred for 1 h at  $-45^\circ\text{C}$  and evaporated under reduced pressure to obtain the crude triflate 29.

A solution of the crude triflate 29, 2,6-lutidine (0.025 mL, 0.215 mmol, Aldrich), and the pyridine 9 in acetone- $d_6$  (1.5 mL, Aldrich) was stored at room temperature for 2 h. The solution was concentrated under reduced pressure to obtain crude pyridinium product:  $^{31}\text{P}$  NMR (acetone- $d_6$ )  $\delta$  25.8; MS (ESI) 852 ( $\text{M}^+$ ).

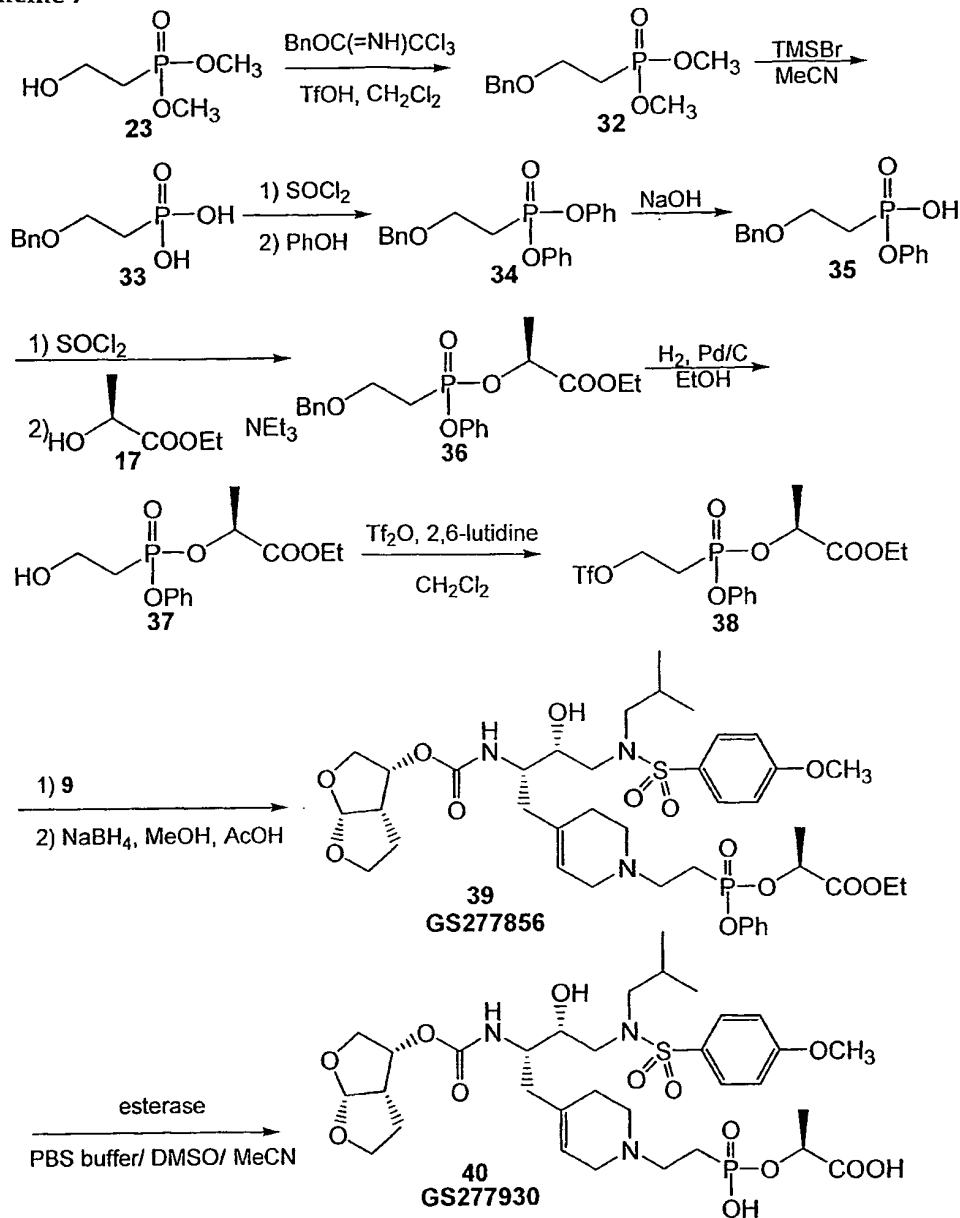
To a solution of the crude pyridinium salt in ethanol (2 mL) was added 7~8 drops of a solution of acetic acid (0.4 mL, Aldrich) in ethanol (2 mL). The solution was stirred at  $0^\circ\text{C}$  as  $\text{NaBH}_4$  (7~8 mg) was added. The solution was maintained to be pH 3-4 by adding the acetic acid solution. More  $\text{NaBH}_4$  and the acetic acid were added until the reduction was completed. After 4 h, the mixture was concentrated and the remaining residue was dissolved in saturated  $\text{NaHCO}_3$  (10 mL). The product was extracted with EtOAc (10 mL x 3), dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. The residue was purified by repeated chromatography on silica gel followed by HPLC purification. Lyophilization of the collected fraction resulted the product 30 (13.5 mg, 26%) as trifluoroacetic acid salt:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d, 2H,  $J = 8.7$  Hz), 7.36 (br, 10H), 7.00 (d, 2H,  $J = 8.7$  Hz), 5.69 (d, 1H,  $J = 5.1$  Hz), 5.41 (br, 1H), 5.13-4.93 (m, 6H), 4.05-2.5 (m, 19H), 3.88 (s, 3H), 2.5-1.9 (m, 5H), 1.90-1.74 (m, 2H), 0.88 (d, 6H,  $J = 6.1$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  25.8; MS (ESI) 856 ( $\text{M}+\text{H}$ ).

#### Example 22

Phosphonic acid 31: A mixture of the dibenzyl phosphonate 30 (9.0 mg, 0.009 mmol) and 10% Pd/C (5.2 mg, Aldrich) in EtOAc (2 mL) and ethanol (0.5 mL) was stirred under  $\text{H}_2$  atmosphere for 3 h at room temperature. After the mixture was filtered through celite, a drop

of trifluoroacetic acid (Aldrich) was added to the filtrate and the filtrate was concentrated to dryness to afford the product **31** (6.3 mg, 86%):  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.76 (d, 2H,  $J = 9.0$  Hz), 7.11 (d, 2H,  $J = 9.0$  Hz), 5.69 (d, 1H,  $J = 5.1$  Hz), 5.54 (br, 1H), 5.09 (br, 1H), 4.05-3.84 (m, 4H), 3.89 (s, 3H), 3.84-3.38 (m, 9H), 3.07 (dd, 2H,  $J = 13.5$  and 8.4 Hz), 2.9-2.31 (m, 5H), 2.31-1.83 (m, 6H), 0.92 (d, 3H,  $J = 6.3$  Hz), 0.85 (d, 3H,  $J = 6.9$  Hz);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  21.6; MS (ESI) 676 (M+H).

Scheme 7

**Example 23**

**Benzylether 32:** A solution of dimethyl hydroxyethylphosphonate (5.0 g, 32.5 mmol, Across) and benzyl 2,2,2-trichloroacetimidate (97.24 mL, 39.0 mmol, Aldrich) in  $\text{CH}_2\text{Cl}_2$  (100 mL) at  $0^\circ\text{C}$  under a nitrogen atmosphere was treated with trifluoromethanesulfonic acid (0.40 mL).

Stirring was performed for three hours at 0°C and the reaction was then allowed to warm to room temperature while stirring continued. The reaction continued for 15 hours, and the reaction mixture was then diluted with dichloromethane, washed with saturated sodium bicarbonate, washed with brine, dried (MgSO<sub>4</sub>), concentrated under reduced pressure and chromatographed on silica gel eluting with a gradient of eluent from 60% EtOAc in hexane to 100% EtOAc to afford 4.5 g, (57%) of the benzyl ether as a colorless liquid. <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 31.5.

#### Example 24

Diacid 33: A solution of benzylether 32 (4.5 g, 18.4 mmol) was dissolved in anhydrous acetonitrile (100mL), chilled to 0°C under a nitrogen atmosphere and treated with TMS bromide (9.73 mL, 74mmol). The reaction mixture was warmed to room temperature and after 15 hours of stirring was concentrated repeatedly with MeOH/water to afford the diacid, which was used directly in the next step. <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 31.9.

#### Example 25

Diphenylphosphonate 34 : Diacid 33 (6.0 g, 27 mmol) was dissolved in toluene and concentrated under reduced pressure three times, dissolved in anhydrous acetonitrile, stirred under a nitrogen atmosphere, and treated with thionyl chloride (20 mL, 270 mmol) by slow addition. The solution was heated to 70°C for two hours, then cooled to room temperature, concentrated and dissolved in anhydrous dichloromethane, chilled to -78°C and treated with phenol (15 g, 162 mmol) and triethylamine (37 mL, 270 mmol). The reaction mixture was warmed to room temperature and stirred for 15 hours, and was then diluted with ice cold dichloromethane, washed with ice cold 1 N. NaOH, washed with ice cold water, dried (MgSO<sub>4</sub>), and concentrated under reduced pressure. The resulting residue was used directly in the next step. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.40-7.16 (d, 15H), 4.55 (s, 2H), 3.98-3.84 (m, 2H), 2.55-2.41 (m, 2H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 22.1.

#### Example 26

Mono acid 35: Monoacid 35 was prepared using conditions analogous to those used to prepare monoacid 16, except that diphenylphosphonate 34 was substituted for benzylether 15.

$^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.38-7.16 (d, 10H), 4.55 (s, 2H), 3.82-3.60 (m, 3H), 2.33-2.21 (m, 2H);  
 $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  29.0.

#### Example 27

- 5 Ethyl lactate phosphonate 36: Ethyl lactate phosphonate 36 was prepared analogously to ethyl lactate phosphonate 18 except monoacid 35 was substituted for monoacid 16.  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  27.0, 25.6.

#### Example 28

- 1 Ethyl lactate phosphonate with free alcohol 37: Ethyl lactate phosphonate with free alcohol 37 was prepared analogously to ethyl lactate phosphonate with free alcohol 19 except that ethyl lactate phosphonate 36 was substituted for ethyl lactate phosphonate 18.  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  28.9, 26.8.

#### Example 29

Triflate 38: A solution of the alcohol 37 (663 mg, 2.19 mmol) and 2,6-lutidine (0.385 mL, 3.31 mmol, Aldrich) in  $\text{CH}_2\text{Cl}_2$  (5 mL) was stirred at  $-45^\circ\text{C}$  as trifluoromethanesulfonic anhydride (0.48 mL, 2.85 mmol, Aldrich) was added. The solution was stirred for 1.5 h at  $-45^\circ\text{C}$ , diluted with ice-cold water (50 mL), and extracted with EtOAc (30 mL x 2). The combined extracts were washed with ice cold water (50 mL), dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure to obtain a crude mixture of two diastereomers (910 mg, 96%, 1:3 ratio):  $^1\text{H}$  NMR (acetone- $d_6$ )  $\delta$  7.48-7.37 (m, 2H), 7.37-7.18 (m, 3H), 5.2-4.95 (m, 3H), 4.3-4.02 (m, 2H), 3.38-3.0 (m, 1H), 3.0-2.7 (m, 2H), 2.1-1.9 (m, 1H), 1.52 (d, 1H), 1.4 (d, 2H), 1.4-1.1 (m, 3H);  $^{31}\text{P}$  NMR (acetone- $d_6$ )  $\delta$  21.8 (0.75P), 20.5 (0.25P).

#### Example 30

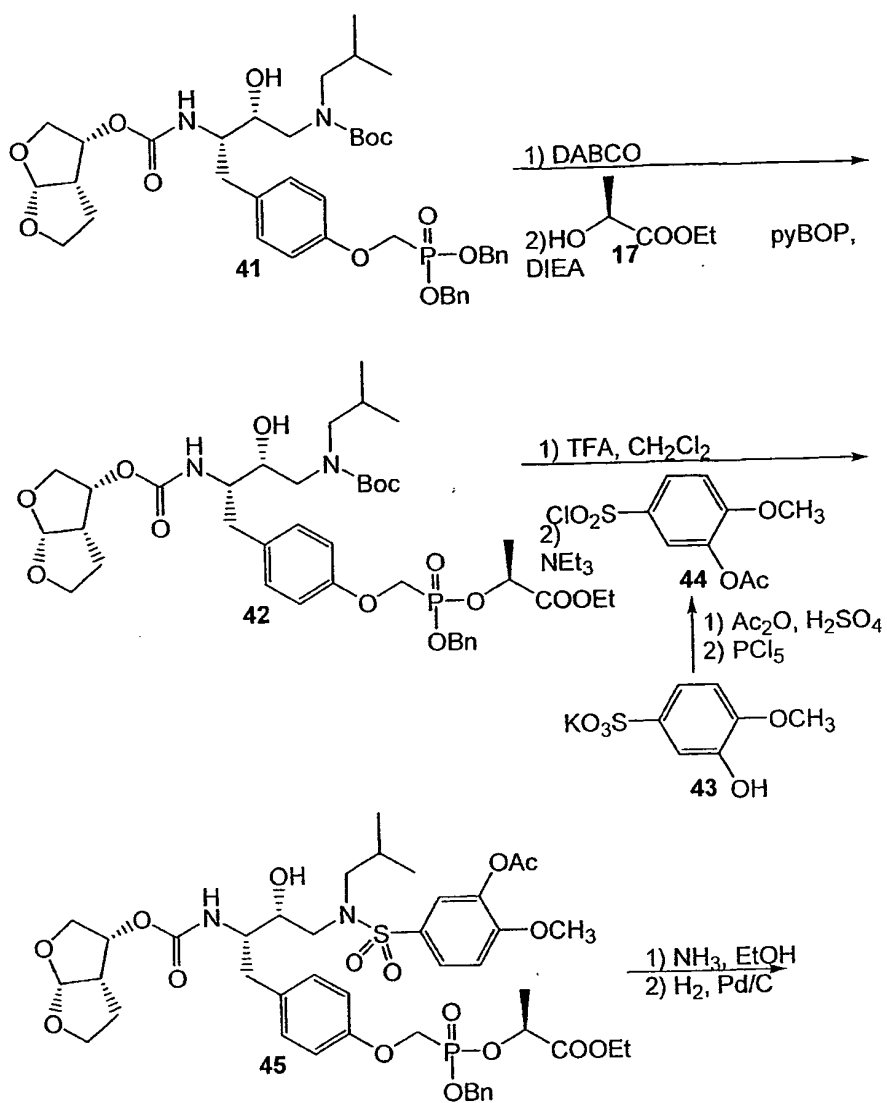
The prodrug 39: A solution of the crude triflate 38 (499 mg, 1.15 mmol) and the pyridine 9 (494 mg, 0.877 mmol) in acetone (5 mL) was stirred at room temperature for 16.5 h. The solution was concentrated under reduced pressure to obtain the crude pyridinium salt. To a solution of the crude pyridinium salt in ethanol (10 mL) was added 5 drops of a solution of acetic acid (1 mL) in ethanol (5 mL). The solution was stirred at  $0^\circ\text{C}$  as  $\text{NaBH}_4$  (~10 mg, Aldrich) was added. The solution was maintained to be pH 3-4 by adding the acetic acid

solution. More  $\text{NaBH}_4$  and the acetic acid were added until the reduction was completed. After 5.5 h, the mixture was concentrated under reduced pressure and the remaining residue was dissolved in ice-cold saturated  $\text{NaHCO}_3$  (50 mL). The product was extracted with ice-cold EtOAc (30 mL x 2) and the combined extracts were washed with 50% saturated  $\text{NaHCO}_3$  (50 mL), dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. The residue was purified by a chromatography on silica gel followed by a chromatography on C18 reverse phase column material. Lyophilization of the collected fraction resulted the product **39** mixture (376 mg, 50%, ~2.5:1 ratio) as trifluoroacetic acid salt:  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}+\text{TFA}$ )  $\delta$  7.78 (d, 2H,  $J = 8.7$  Hz), 7.52-7.42 (m, 2H); 7.37-7.22 (m 3H), 7.10 (d, 2H,  $J = 8.7$  Hz), 5.78 (d, 1H,  $J = 9.0$  Hz), 5.64 (m, 1H), 5.50 (br, 1H), 5.08 (m, 2H), 4.31-4.12 (m, 2H), 4.04-3.42 (m, 11H), 3.90 (s, 3H), 3.29 (m, 2H), 3.23-3.16 (m, 1H), 3.08-2.78 (m, 6H), 2.76-2.27 (m, 5H), 2.23-2.11 (m, 1H), 2.08-1.77 (m, 3H), 1.58 (d, 0.9H,  $J = 7.2$  Hz), 1.45 (d, 2.1H,  $J = 6.6$  Hz), 1.32-1.20 (m, 3H), 0.95 - 0.84 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{CN}+\text{TFA}$ )  $\delta$  24.1 and 23.8, 22.2 and 22.1; MS (ESI) 852 (M+H).

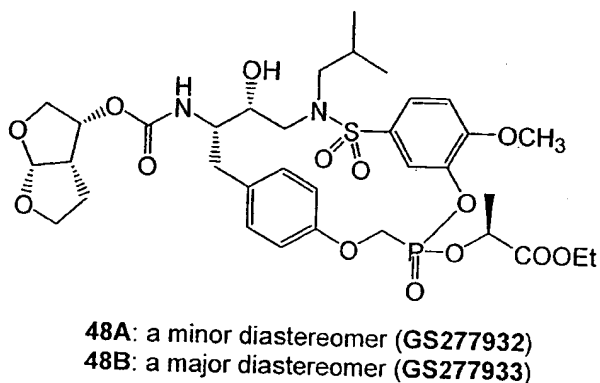
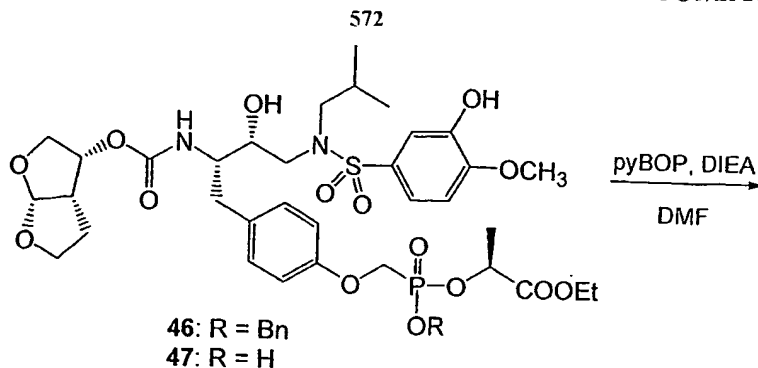
#### Example 31

Metabolite 40: To a solution of the prodrug **39** (35.4 mg, 0.037 mmol) in DMSO (0.35 mL) and acetonitrile (0.70 mL) was added 0.1 M PBS buffer (10.5 mL) mixed thoroughly to result a suspension. To the suspension was added porcine liver esterase suspension (0.175 mL, EC3.1.1.1, Sigma). After the suspension was stored in 37°C for 6.5 h, the mixture was filtered through 0.45  $\mu\text{m}$  membrane filter and the filtrate was purified by HPLC. The collected fraction was lyophilized to result the product **40** as trifluoroacetic acid salt (28.8 mg, 90%):  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )  $\delta$  7.96 (d, 2H,  $J = 8.7$  Hz), 7.32 (d, 2H,  $J = 8.7$  Hz), 5.89 (d, 1H,  $J = 5.1$  Hz), 5.66 (br, 1H), 5.27 (m, 1H), 4.97 (m, 1H), 4.23-4.12 (m, 2H), 4.08 (s, 3H), 4.06-3.10 (m, 14H), 3.03 (dd, 1H,  $J = 14.1$  and 6.6 Hz), 2.78-1.97 (m, 9H), 1.66 (d, 3H,  $J = 6.9$  Hz), 1.03 (d, 3H,  $J = 7.5$  Hz), 1.01 (d, 3H,  $J = 6.9$  Hz);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{CN}+\text{TFA}$ )  $\delta$  20.0, 19.8; MS (ESI) 748 (M+H).

Scheme 8





**Example 32**

Compound 42: The dibenzyl phosphonate **41** (947 mg, 1.21 mmol) was treated with DABCO (140.9 mg, 1.26 mmol, Aldrich) in 4.5 mL toluene to obtain the monoacid (890 mg, 106%). The crude monoacid (890 mg) was dried by evaporation with toluene twice and dissolved in DMF (5.3 mL) with ethyl (*S*)-lactate (0.3 mL, 2.65 mmol, Aldrich) and pyBOP (945 mg, 1.82 mmol, Aldrich) at room temperature. After diisopropylethylamine (0.85 mL, 4.88 mmol, Aldrich) was added, the solution was stirred at room temperature for 4 h and concentrated under reduced pressure to a half volume. The resulting solution was diluted with 5% aqueous HCl (30 mL) and the product was extracted with EtOAc (30 mL x 3). After the combined extracts were dried (MgSO<sub>4</sub>) and concentrated, the residue was chromatographed on silica gel to afford the compound **42** (686 mg, 72%) as a mixture of two diastereomers (2:3 ratio): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.46-7.32 (m, 5H), 7.13 (d, 2H, *J* = 8.1 Hz), 6.85 (t, 2H, *J* = 8.1 Hz), 5.65 (m, 1H), 5.35-4.98 (m, 4H), 4.39 (d, 0.8H, *J* = 10.2 Hz), 4.30-4.14 (m, 3.2H), 3.98 (dd, 1H, *J* = 9.3 and 6.0 Hz), 3.92-3.78 (m, 3H), 3.78-3.55 (m, 3H), 3.16-2.68 (m, 6H), 1.85 (m, 1H), 1.74-1.55 (m, 2H), 1.56 (d, 1.8H, *J* = 7.2 Hz), 1.49 (d, 1.2H), 1.48 (s, 9H), 1.30-1.23 (m, 3H), 0.88 (d, 3H, *J* = 6.3 Hz), 0.87 (d, 3H, *J* = 6.3 Hz); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.8 (0.4P), 19.5 (0.6P); MS (ESI) 793 (M+H).

Example 33

Compound 45: A solution of compound **42** (101 mg, 0.127 mmol) and trifluoroacetic acid (0.27 mL, 3.5 mmol, Aldrich) in  $\text{CH}_2\text{Cl}_2$  (0.6 mL) was stirred at 0°C for 3.5 h and concentrated under reduced pressure. The resulting residue was dried in vacuum to result the crude amine as TFA salt.

A solution of the crude amine salt and triethylamine (0.072 mL, 0.52 mmol, Aldrich) in  $\text{CH}_2\text{Cl}_2$  (1 mL) was stirred at 0°C as the sulfonyl chloride **42** (37 mg, 0.14 mmol) was added. After the solution was stirred at 0°C for 4 h and 0.5 h at room temperature, the reaction mixture was diluted with saturated  $\text{NaHCO}_3$  (20 mL) and extracted with EtOAc (20 mL x 1; 15 mL x 2). The combined organic fractions were washed with saturated NaCl solution, dried ( $\text{MgSO}_4$ ), and concentrated under reduced pressure. Purification by chromatography on silica gel provided the sulfonamide **45** (85 mg, 72%) as a mixture of two diastereomers (~1:2 ratio):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.45-7.31 (m, 7H), 7.19 (d, 1H,  $J = 8.4$  Hz), 7.12 (d, 2H,  $J = 7.8$  Hz), 6.85 (m, 2H), 5.65 (d, 1H,  $J = 5.4$  Hz), 5.34-5.16 (m, 2H), 5.13-4.97 (m, 2H), 4.97-4.86 (m, 1H), 4.38 (d, 0.7H,  $J = 10.8$  Hz), 4.29-4.12 (m, 3.3H), 3.96 (dd, 1H,  $J = 9.3$  and 6.3 Hz), 3.89 (s, 3H), 3.92-3.76 (m, 3H), 3.76-3.64 (m, 2H), 3.64-3.56 (br, 1H), 3.34-3.13 (m, 1H), 3.11-2.70 (m, 6H), 2.34 (s, 3H), 1.86 (m, 1H,  $J = 7.0$  Hz), 1.75-1.58 (m, 2H), 1.56 (d, 2H,  $J = 7.2$  Hz), 1.49 (d, 1H,  $J = 7.2$  Hz), 1.29-1.22 (m, 3H), 0.94 (d, 3H,  $J = 6.6$  Hz), 0.90 (d, 3H,  $J = 6.9$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.7 (0.3P), 19.5 (0.7P); MS (ESI) 921 (M+H).

Example 34

Compound 46: Compound **45** (257 mg, 0.279 mmol) was stirred in a saturated solution of ammonia in ethanol (5 mL) at 0°C for 15 min and the solution was concentrated under reduced pressure. Purification of the residue by chromatography on silica gel provided compound **46** (2.6 mg, 84%):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.48-7.34 (m, 4H), 7.22-7.05 (m, 5H), 7.01 (d, 1H,  $J = 8.1$  Hz), 6.87-6.80 (m, 2H), 5.68 (d, 1H,  $J = 4.8$  Hz), 5.32 (dd, 1.3H,  $J = 8.7$  and 1.8 Hz), 5.22 (d, 0.7H,  $J = 9.0$  Hz), 5.11-5.00 (m, 3H), 4.47-4.14 (m, 4H), 4.00 (dd, 1H,  $J = 9.9$  and 6.6 Hz), 3.93 (s, 3H), 3.95-3.63 (m, 5H), 3.07-2.90 (m, 4H), 2.85-2.75 (m, 1H), 2.75-2.63 (m, 2H), 1.88-1.67 (m, 3H), 1.65-1.55 (m, 2H), 1.57 (d, 2H,  $J = 6.9$  Hz), 1.50 (d, 1H,  $J = 7.2$  Hz), 1.31-1.20 (m, 3H), 0.95 (d, 3H,  $J = 6.6$  Hz), 0.88 (d, 3H,  $J = 6.3$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.7 (0.3P), 19.6 (0.7P); MS (ESI) 879 (M+H).

Example 35

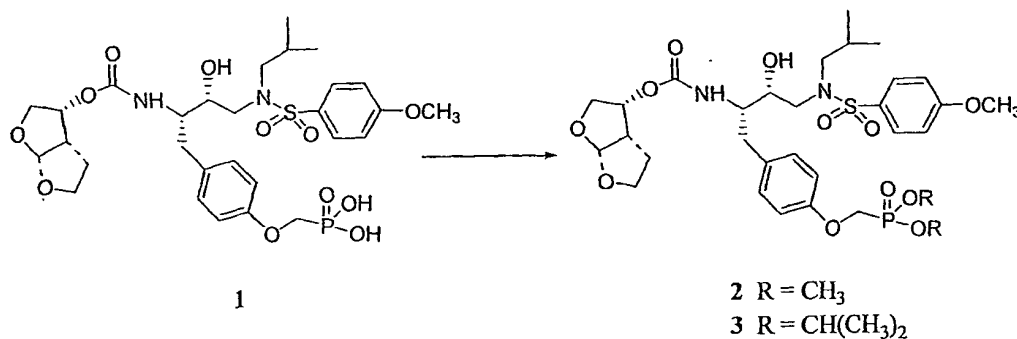
Compound 47: A mixture of compound 46 (176 mg, 0.200 mmol) and 10% Pd/C (9.8 mg, Aldrich) in EtOAc (4 mL) and ethanol (1 mL) was stirred under H<sub>2</sub> atmosphere for 3 h at room temperature. After the mixture was filtered through celite, the filtrate was concentrated to dryness to afford compound 47 (158 mg, 100%) as white powder: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.30-7.16 (m, 2H), 7.12 (d, 2H, *J* = 7.5 Hz), 7.01 (d, 1H, *J* = 7.8 Hz), 6.84 (d, 2H, *J* = 7.5 Hz), 5.66 (d, 1H, *J* = 4.5 Hz), 5.13-4.97 (m, 2H), 4.38-4.10 (m, 4H), 3.93 (s, 3H), 4.02-3.66 (m, 6H), 3.13-2.69 (m, 7H), 1.96-1.50 (m, 3H), 1.57 (d, 3H, *J* = 6.6 Hz), 1.26 (t, 3H, *J* = 7.2 Hz), 0.93 (d, 3H, *J* = 6.0 Hz), 0.88 (d, 3H, *J* = 6.0 Hz); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.1; MS (ESI) 789 (M+H).

Example 36

Compound 48A and 48B: A solution of pyBOP (191 mg, 0.368 mmol, Aldrich) and diisopropylethylamine (0.1 mL, 0.574 mmol, Aldrich) in DMF (35 mL) was stirred at room temperature as a solution of compound 47 (29 mg, 0.036 mmol) in DMF (5.5 mL) was added over 16 h. After addition, the solution was stirred at room temperature for 3 h and concentrated under reduced pressure. The residue was dissolved in ice-cold water and extracted with EtOAc (20 mL x 1; 10 mL x 2). The combined extracts were dried (MgSO<sub>4</sub>) and concentrated under reduced pressure. The residue was purified by chromatography on silica gel followed by preparative TLC gave two isomers of structure 48 (1.0 mg, 3.6% and 3.6 mg, 13%). Isomer 48A: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.39 (m, 1H), 7.12 (br, 1H), 7.01 (d, 2H, *J* = 8.1 Hz), 6.98 (br, 1H), 6.60 (d, 2H, *J* = 8.1 Hz), 5.75 (d, 1H, *J* = 5.1 Hz), 5.37-5.28 (m, 2H), 5.18 (q, 1H, *J* = 8.7 Hz), 4.71 (dd, 1H, *J* = 14.1 and 7.5 Hz), 4.29 (m, 3H), 4.15-4.06 (m, 1H), 3.99 (s, 3H), 4.05-3.6 (m, 5H), 3.35 (m, 1H), 3.09 (br, 1H), 2.90-2.78 (m, 3H), 2.2-2.0 (m, 3H), 1.71 (d, 3H, *J* = 6.6 Hz), 1.34 (t, 3H, *J* = 6.9 Hz), 1.01 (d, 3H, *J* = 6.3 Hz), 0.95 (d, 3H, *J* = 6.3 Hz); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.8; MS (ESI) 793 (M+Na); isomer 48B: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.46 (d, 1H, *J* = 9.3 Hz), 7.24 (br, 1H), 7.00 (d, 2H, *J* = 8.7 Hz), 6.91 (d, 1H, *J* = 8.7 Hz), 6.53 (d, 2H, *J* = 8.7 Hz), 5.74 (d, 1H, *J* = 5.1 Hz), 5.44 (m, 1H), 5.35 (d, 1H, *J* = 9.0 Hz), 5.18 (q, 1H, *J* = 7.2 Hz), 4.68 (dd, 1H, *J* = 14.4 and 6.3 Hz), 4.23 (m, 3H), 4.10 (m, 1H), 4.04 (s, 3H), 3.77-4.04 (m, 6H), 3.46 (dd, 1H, *J* = 12.9 and 11.4 Hz), 3.08 (br, 1H), 2.85 (m, 2H), 2.76 (dd, 1H, *J* = 12.9 and 4.8 Hz), 1.79-2.11 (m, 3H), 1.75 (d, 3H, *J* = 6.6 Hz), 1.70 (m, 2H),

1.27 (t, 3H,  $J = 6.9$  Hz), 1.01 (d, 3H,  $J = 6.6$  Hz), 0.93 (d, 3H,  $J = 6.6$  Hz);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.4; MS (ESI) 793 ( $\text{M} + \text{Na}$ ).

### Example 1



#### Example 1A

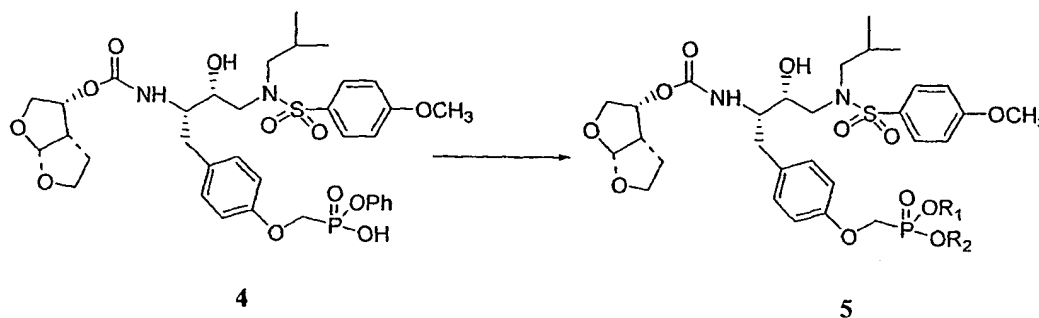
Dimethylphosphonic ester 2 (R = CH<sub>3</sub>): To a flask was charged with phosphonic acid 1 (67 mg, 0.1 mmol), methanol (0.1 mL, 2.5 mmol) and 1, 3-dicyclohexylcarbodiimide (83 mg, 0.4 mmol), then pyridine (1 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60 –70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol/CH<sub>2</sub>Cl<sub>2</sub>, 1% to 7%) to give 2 (39 mg, 56 %) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71(d,  $J = 8.7$  Hz, 2H), 7.15 (d,  $J = 8.7\text{Hz}$ , 2H), 7.00 (d,  $J = 8.7$  Hz, 2H), 6.87 (d,  $J = 8.7$  Hz, 2H), 5.65 (d,  $J = 5.1$  Hz, 1H), 5.10-4.92 (m, 4H), 4.26 (d,  $J = 9.9$  Hz, 2H), 3.96 -3.65 (m overlapping s, 15H), 3.14-2.76 (m, 7H), 1.81-1.55 (m, 3H), 0.91 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  21.7; MS (ESI) 723 ( $\text{M} + \text{Na}$ ).

#### Example 1B

Diisopropylphosphonic ester 3 (R = CH (CH<sub>3</sub>)<sub>2</sub>) was synthesized in the same manner in 60% yield.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71(d,  $J = 8.7$  Hz, 2H), 7.15 (d,  $J = 8.7\text{Hz}$ , 2H), 7.15 (d,  $J = 8.7$  Hz, 2H), 6.99 (d,  $J = 8.7$  Hz, 2H), 6.87 (d,  $J = 8.7$  Hz, 2H), 5.66 (d,  $J = 5.1$  Hz, 1H), 5.08-4.92 (m, 3H), 4.16 (d,  $J = 10.5$  Hz, 2H), 3.98 -3.68 (m overlapping s, 9H), 3.16-2.78 (m, 7H),

1.82-1.56 (m, 3H), 1.37 (t,  $J = 6.3$  Hz, 6H), 0.93 (d,  $J = 6.6$  Hz, 3H), 0.88 (d,  $J = 6.6$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.3; MS (ESI) 779 ( $\text{M}+\text{Na}$ ).

### Example 2



| Compound | R <sub>1</sub> | R <sub>2</sub>         |
|----------|----------------|------------------------|
| 5a       | OPh            | mix-Hba-Et             |
| 5b       | OPh            | ( <i>S</i> )-Hba-Et    |
| 5c       | OPh            | ( <i>S</i> )-Hba-tBu   |
| 5d       | OPh            | ( <i>S</i> )-Hba-EtMor |
| 5e       | OPh            | ( <i>R</i> )-Hba-Et    |

### Example 2A

Monolactate **5a** ( $\text{R}_1 = \text{OPh}$ ,  $\text{R}_2 = \text{Hba-Et}$ ): To a flask was charged with monophenyl phosphonate **4** (250 mg, 0.33 mmol), 2-hydroxy-*n*-butyric acid ethyl ester (145 mg, 1.1 mmol) and 1, 3-dicyclohexylcarbodiimide (226 mg, 1.1 mmol), then pyridine (2.5 mL) was added under  $\text{N}_2$ . The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with  $\text{NH}_4\text{Cl}$ , brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel ( $\text{EtOAc}/\text{CH}_2\text{Cl}_2$ , 1:1) to give **5a** (150 mg, 52 %) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.70 (d,  $J = 8.7$  Hz, 2H), 7.37-7.19 (m, 5H), 7.14 (d,  $J = 8.7$  Hz, 2H), 7.00 (d,  $J = 8.7$  Hz, 2H), 6.91 (d,  $J = 8.7$  Hz, 1H), 6.86 (d,  $J = 8.7$  Hz, 1H), 5.65 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96-3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.21 (m, 3H), 1.04-0.86 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.5 and 15.1; MS (ESI) 885 ( $\text{M}+\text{Na}$ ).

Example 2B

Monolactate **5b** (R1 = OPh, R2 = (S)-Hba-Et): To a flask was charged with monophenyl phosphonate **4** (600 mg, 0.8 mmol), (S)-2-hydroxy-n-butyric acid ethyl ester (317 mg, 2.4 mmol) and 1, 3-dicyclohexylcarbodiimide (495 mg, 2.4 mmol), then pyridine (6 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH<sub>2</sub>Cl<sub>2</sub>, 1:1) to give **5b** (360 mg, 52 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (d, J = 8.7 Hz, 2H), 7.37-7.19 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96 -3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.23 (m, 3H), 1.04-0.86 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.5 and 15.2; MS (ESI) 885 (M+Na).

Example 2C

Monolactate **5c**(R1 = OPh, R2 = (S)-Hba-tBu): To a flask was charged with monophenyl phosphonate **4** (120 mg, 0.16 mmol), tert-butyl (S)-2-hydroxybutyrate (77 mg, 0.48 mmol) and 1, 3-dicyclohexylcarbodiimide (99 mg, 0.48 mmol), then pyridine (1 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH<sub>2</sub>Cl<sub>2</sub>, 1:1) to give **5c** (68 mg, 48 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (d, J = 8.7 Hz, 2H), 7.37-7.19 (m, 5H), 7.14 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.93 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.64 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96 -3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.44 (d, J = 11 Hz, 9H), 1.04-0.86 (m, 9H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.5 and 15.2; MS (ESI) 913 (M+Na).

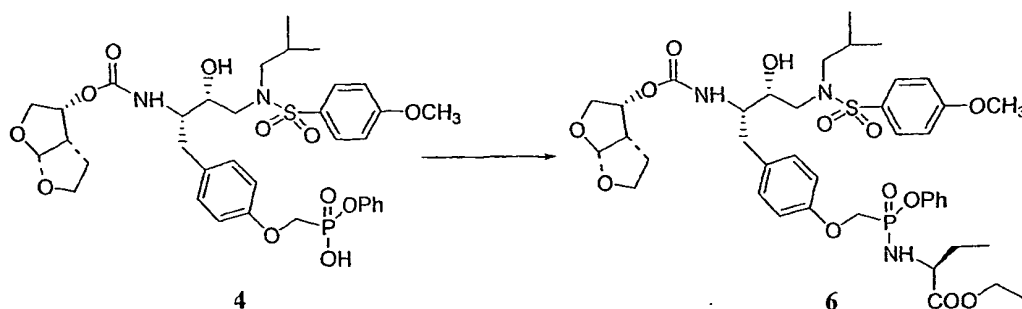
Example 2D

Monolactate **5d** (R1 = OPh, R2 = (S)-Lac-EtMor): To a flask was charged with monophenyl phosphonate **4** (188 mg, 0.25 mmol), (S)-lactate ethylmorpholine ester (152 mg, 0.75 mmol)

and 1, 3-dicyclohexylcarbodiimide (155 mg, 0.75 mmol), then pyridine (2mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was washed with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol/CH<sub>2</sub>Cl<sub>2</sub>, 1:9) to give **5d** (98 mg, 42 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.34-7.20 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.21-4.99 (m, 3H), 4.57-4.20 (m, 4H), 3.97 -3.63 (m overlapping s, 13H), 3.01-2.44 (m, 13H), 1.85-1.50 (m, 6H), 0.92 (d, J = 6.5 Hz, 3H), 0.88 (d, J = 6.5, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.4 and 15.3; MS (ESI) 934(M).

#### Example 2E

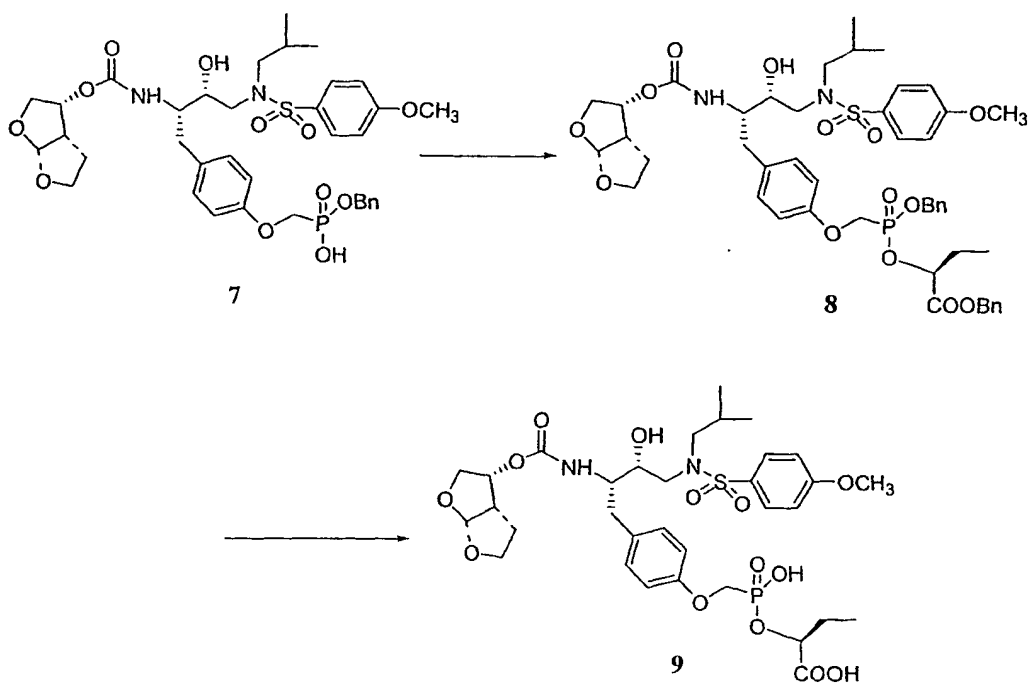
Monolactate **5e** (R1 = OPh, R2 = (*R*)-Hba-Et): To a flask was charged with monophenyl phosphonate **4** (600 mg, 0.8 mmol), (*R*)-2-hydroxy-*n*-butyric acid ethyl ester (317 mg, 2.4 mmol) and 1, 3-dicyclohexylcarbodiimide (495 mg, 2.4 mmol), then pyridine (6 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOAc/CH<sub>2</sub>Cl<sub>2</sub>, 1:1) to give **5e** (345 mg, 50 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.70 (d, J = 8.7 Hz, 2H), 7.37-7.19 (m, 5H), 7.15 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 8.7 Hz, 1H), 6.86 (d, J = 8.7 Hz, 1H), 5.65 (m, 1H), 5.10-4.95 (m, 3H), 4.57-4.39 (m, 2H), 4.26 (m, 2H), 3.96 -3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.23 (m, 3H), 1.04-0.86 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.5 and 15.1; MS (ESI) 885 (M+Na).



### Example 3

**Monoamidate 6:** To a flask was charged with monophenyl phosphonate **4** (120 mg, 0.16 mmol), L-alanine butyric acid ethyl ester hydrochloride (160 mg, 0.94 mmol) and 1, 3-dicyclohexylcarbodiimide (132 mg, 0.64 mmol), then pyridine (1 mL) was added under N<sub>2</sub>. The resulted mixture was stirred at 60–70°C for 2 h, then cooled to room temperature and diluted with ethyl acetate. The mixture was filtered and the filtrate was evaporated. The residue was diluted with ethyl acetate and the combined organic phase was washed with NH<sub>4</sub>Cl, brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol/CH<sub>2</sub>Cl<sub>2</sub>, 1:9) to give **6** (55 mg, 40 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.72 (d, J = 8.7 Hz, 2H), 7.37-7.23 (m, 5H), 7.16 (d, J = 8.7 Hz, 2H), 7.00 (d, J = 8.7 Hz, 2H), 6.90-6.83 (m, 2H), 5.65 (d, J = 5.1 Hz, 1H), 5.10-4.92 (m, 3H), 4.28 (m, 2H), 3.96 -3.68 (m overlapping s, 9H), 3.15-2.77 (m, 7H), 1.81-1.55 (m, 5H), 1.23 (m, 3H), 1.04-0.86 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.7 and 19.6; MS (ESI) 884(M+Na).



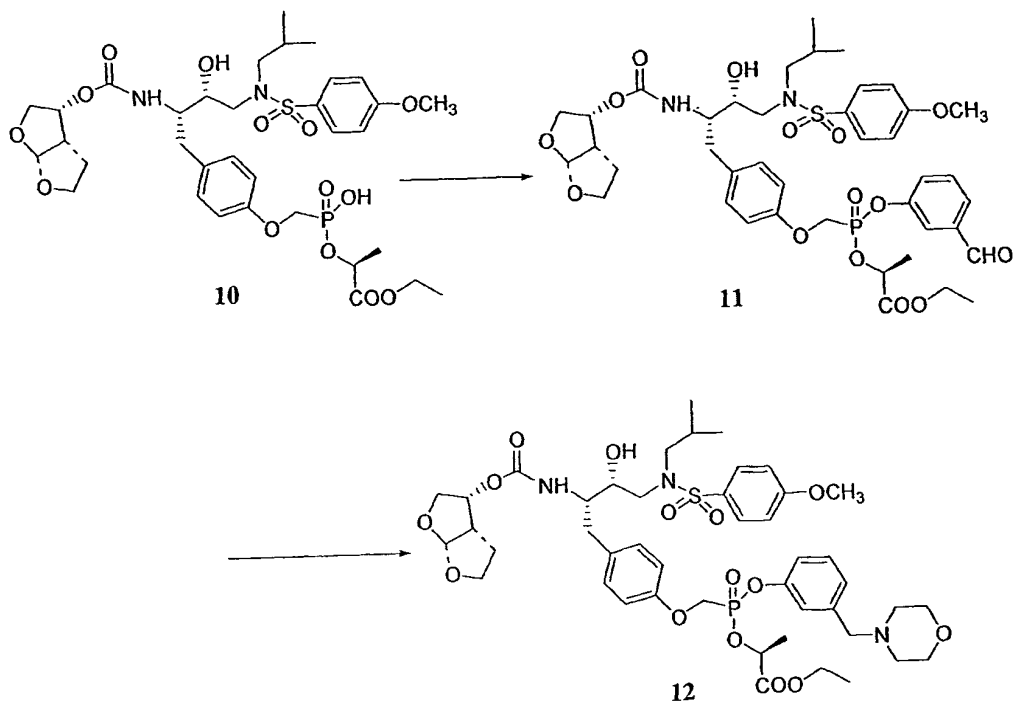
**Example 4A**

Compound 8: To a stirred solution of monobenzyl phosphonate 7 (195 mg, 0.26mmol) in 1 mL of DMF at room temperature under N<sub>2</sub> was added benzyl-(s)-lactate (76 mg, 0.39 mmol) and PyBOP (203 mg, 0.39mmol), followed by DIEA (181μL, 1 mmol). After 3 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) to give 8 (120 mg, 50%) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.71 (d, J = 8.7 Hz, 2H), 7.38-7.34 (m, 5H), 7.12 (d, J = 8.7 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 6.81(d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.4 Hz, 1H), 5.24-4.92 (m, 7H), 4.28 (m, 2H), 3.96 -3.67 (m overlapping s, 9H), 3.16-2.76 (m, 7H), 1.95-1.62 (m, 5H), 0.99-0.87 (m, 9H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 21.0 and 19.7; MS (ESI) 962 (M+Na).

**Example 4B**

Compound 9: A solution of compound 8 (100 mg) was dissolved in EtOH/ EtOAc (9 mL/ 3mL), treated with 10 % Pd/C (10 mg) and was stirred under H<sub>2</sub> atmosphere (balloon) for 1.5 h. The catalyst was removed by filtration through celite. The filtered was evaporated under reduced pressure, the residue was triturated with ether and the solid was collected by filtration to afford the compound 9 (76mg, 94%) as a white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.76 (d, J = 8.7 Hz, 2H), 7.18 (d, J = 8.7 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 5.59 (d, J = 5.4 Hz, 1H), 5.03-4.95 (m, 2H), 4.28 (m, 2H), 3.90 -3.65 (m overlapping s,

9H), 3.41 (m, 2H), 3.18-2.78 (m, 5H), 2.44 (m, 1H), 1.96 (m, 3H), 1.61 (m, 2H), 1.18 (m, 3H), 0.93 (d,  $J = 6.3$  Hz, 3H), 0.87 (d,  $J = 6.3$  Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  18.3; MS (ESI) 782 ( $\text{M}+\text{Na}$ ).



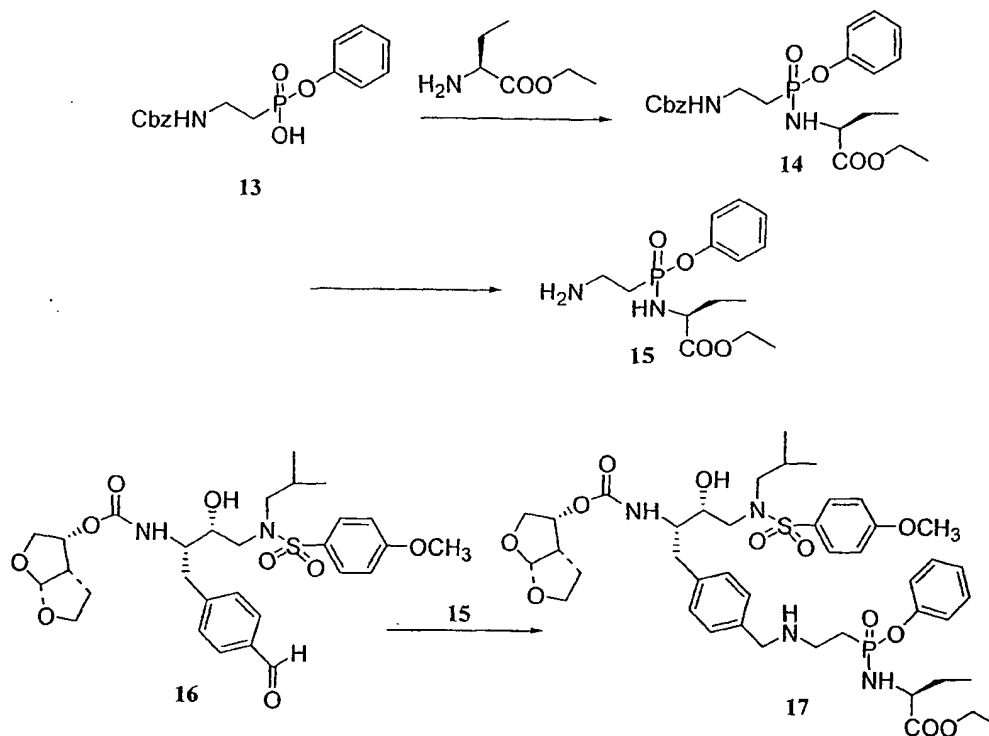
#### Example 5A

**Compound 11:** To a stirred solution of compound **10** (1 g, 1.3mmol) in 6 mL of DMF at room temperature under  $\text{N}_2$  was added 3-hydroxybenzaldehyde (292 mg, 2.6 mmol) and PyBOP (1 g, 1.95mmol), followed by DIEA (0.9 mL, 5.2 mmol). After 5 h, the solvent was removed under reduced pressure, and the resulting crude mixture was purified by chromatography on silica gel (ethyl acetate/hexane 1:1) to give **11** (800 mg, 70%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  9.98 (s, 1H), 7.79-6.88 (m, 12H), 5.65 (m, 1H), 5.21-4.99 (m, 3H), 4.62-4.16 (m, 4H), 3.99-3.61 (m overlapping s, 9H), 3.11-2.79 (m, 5H), 1.85-1.53 (m, 6H), 1.25 (m, 3H), 0.90 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.9 and 15.9; MS (ESI) 899 ( $\text{M}+\text{Na}$ ).

#### Example 5B

**Compound 12:** To a stirred solution of compound **11** (920 mg, 1.05 mmol) in 10 mL of ethyl acetate at room temperature under  $\text{N}_2$  was added morpholine (460 mg, 5.25 mmol) and acetic

acid (0.25 mL, 4.2 mmol), followed by sodium cyanoborohydride (132 mg, 2.1 mmol). After 20h, the solvent was removed under reduced pressure, and the residue was diluted with ethyl acetate and the combined organic phase was washed with  $\text{NH}_4\text{Cl}$ , brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel (isopropanol /  $\text{CH}_2\text{Cl}_2$ , 6%) to give **12** (600 mg, 60%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d,  $J = 8.7$  Hz, 2H), 7.27 (m, 4H), 7.15 (d,  $J = 8.7$  Hz, 2H), 6.95 (d,  $J = 8.7$  Hz, 2H), 6.89 (m, 2H), 5.65 (m, 1H), 5.21-5.02 (m, 3H), 4.58-4.38 (m, 2H), 4.21-4.16 (m, 2H), 3.99-3.63 (m overlapping s, 15H), 3.47 (s, 2H), 3.18-2.77 (m, 7H), 2.41 (s, 4H), 1.85-1.53 (m, 6H), 1.25 (m, 3H), 0.90 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4 and 15.2; MS (ESI) 971 ( $\text{M}+\text{Na}$ ).



#### Example 6A

**Compound 14:** To a stirred solution of compound **13** (1 g, 3 mmol) in 30 mL of acetonitrile at room temperature under  $\text{N}_2$  was added thionyl chloride (0.67 mL, 9 mmol). The resulted mixture was stirred at 60-70°C for 0.5 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 30 mL of DCM, followed by DIEA (1.7 mL, 10 mmol), L-alanine butyric acid ethyl ester hydrochloride (1.7 g, 10 mmol) and TEA (1.7 mL, 12 mmol). After 4h at room temperature, the solvent was removed under

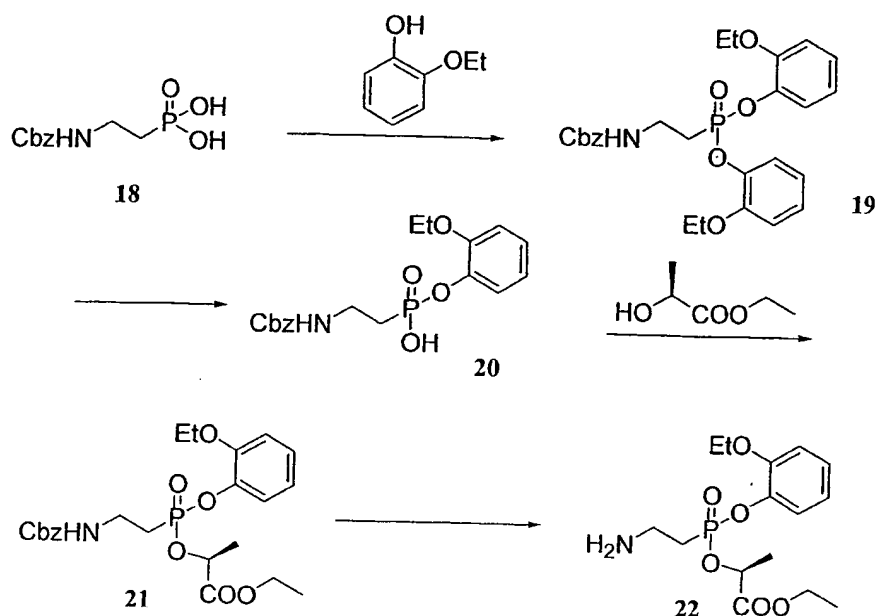
reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel (Hexane/EtOAc 1:1) to give **14** (670 mg, 50%) as a yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.33-7.11 (m, 10H), 5.70 (m, 1H), 5.10 (s, 2H), 4.13-3.53 (m, 5H), 2.20-2.10 (m, 2H), 1.76-1.55 (m, 2H), 1.25-1.19 (m, 3H), 0.85-0.71 (m, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  30.2 and 29.9; MS (ESI) 471 (M+Na).

#### Example 6B

**Compound 15:** A solution of compound **14** (450mg) was dissolved in 9 mL of EtOH, then 0.15 mL of acetic acid and 10 % Pd/C (90 mg) was added. The resulted mixture was stirred under  $\text{H}_2$  atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **15** (300mg, 95%) as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.29-7.12 (m, 5H), 4.13-3.53 (m, 5H), 2.20-2.10 (m, 2H), 1.70-1.55 (m, 2H), 1.24-1.19 (m, 3H), 0.84-0.73(m, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  29.1 and 28.5; MS (ESI) 315 (M+1).

#### Example 6C

**Monoamidate 17:** To a stirred solution of compound **16** (532 mg, 0.9 mmol) in 4 mL of 1,2-dichloroethane was added compound **15** (300 mg, 0.96 mmol) and  $\text{MgSO}_4$  (50 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (1.3 mL, 23 mmol) and sodium cyanoborohydride (1.13 g, 18 mmol) were added. The reaction mixture was stirred at room temperature for 1 h under argon. Then aqueous  $\text{NaHCO}_3$  (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH / EtOAc, 1/9) to give **17** (600 mg, 60%) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (d,  $J$  = 8.7 Hz, 2H), 7.33-7.13 (m, 9H), 7.00 (d,  $J$  = 8.7 Hz, 2H), 5.65 (d,  $J$  = 5.4 Hz, 1H), 5.11-4.98 (m, 2H), 4.22 -3.68 (m overlapping s, 15H), 3.20-2.75 (m, 9H), 2.21-2.10 (m, 2H), 1.88-1.55(m, 5H), 1.29-1.19 (m, 3H), 0.94-0.70 (m, 9H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  31.8 and 31.0; MS (ESI) 889 (M).

Example 7Example 7A

**Compound 19:** To a stirred solution of compound 18 (3.7 g, 14.3 mmol) in 70 mL of acetonitrile at room temperature under N<sub>2</sub> was added thionyl chloride (6.3 mL, 86 mmol). The resulted mixture was stirred at 60-70°C for 2 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 150 mL of DCM, followed by TEA (12 mL, 86 mmol) and 2-ethoxyphenol (7.2 mL, 57.2 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with ethyl acetate and washed with brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM/EtOAc 9:1) to give **19** (4.2 g, 60%) as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.32-6.83 (m, 13H), 5.22 (m, 1H), 5.12 (s, 2H), 4.12-3.73 (m, 6H), 2.52-2.42 (m, 2H), 1.41-1.37 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 25.4; MS (ESI) 522 (M+Na).

Example 7B

**Compound 20:** A solution of compound 19 (3 g, 6 mmol) was dissolved in 70 mL of acetonitrile at 0°C, then 2N NaOH (12 mL, 24 mmol) was added dropwisely. The reaction mixture was stirred at room temperature for 1.5 h. Then the solvent was removed under

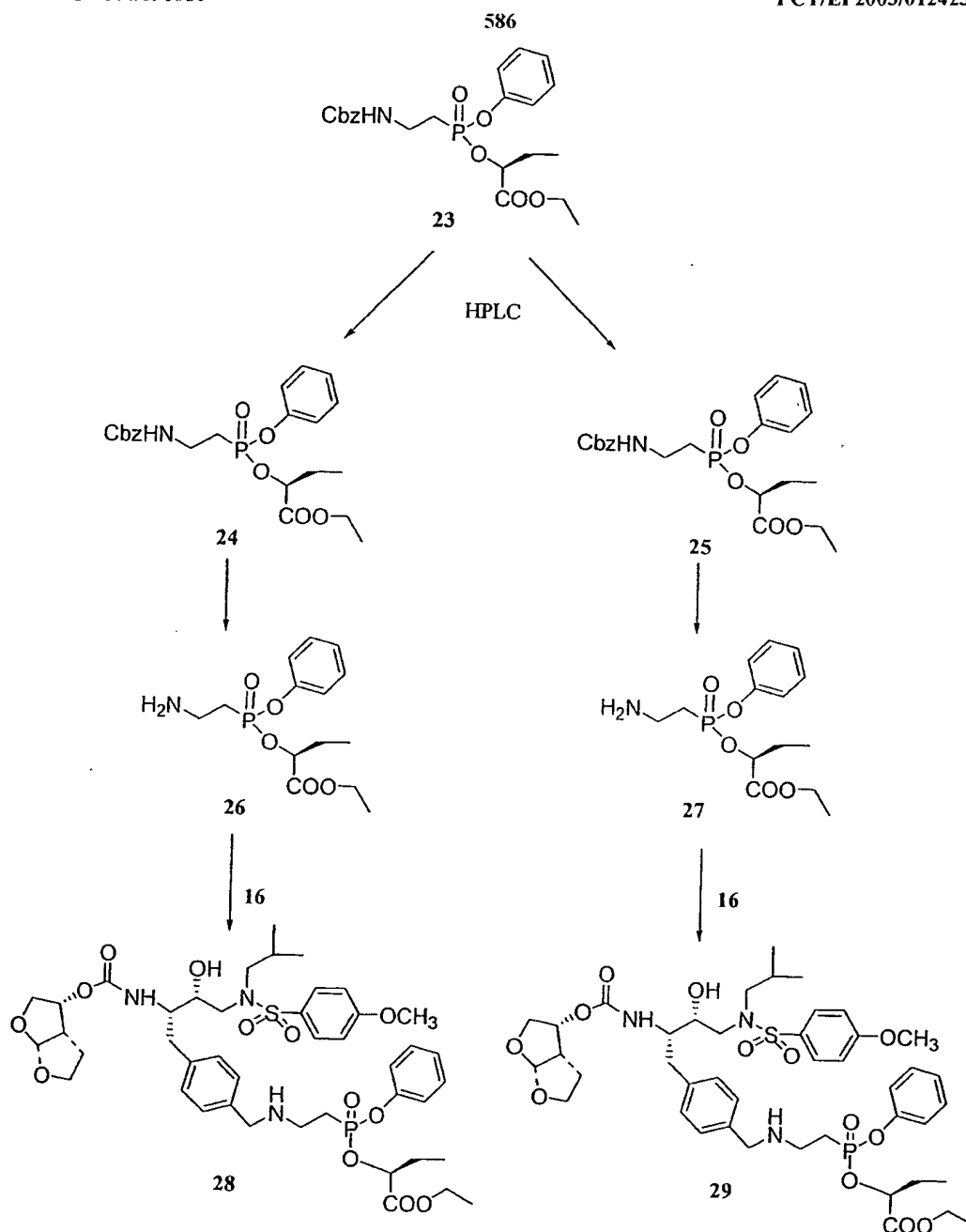
reduced pressure, and the residue diluted with water and extracted with ethyl acetate. The aqueous layer was acidified with conc. HCl to pH = 1, then extracted with ethyl acetate, combined the organic layer and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated to give compound **20** (2 g, 88%) as a off-white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.33-6.79 (m, 9H), 5.10 (s, 2H), 4.12-3.51 (m, 6H), 2.15-2.05 (m, 2H), 1.47-1.33 (m, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 30.5; MS (ESI) 380 (M+1).

#### Example 7C

Compound **21**: To a stirred solution of compound **20** (1 g, 2.6 mmol) in 20 mL of acetonitrile at room temperature under N<sub>2</sub> was added thionyl chloride (1.1 mL, 15.6 mmol). The resulted mixture was stirred at 60-70°C for 45 min. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 25 mL of DCM, followed by TEA (1.5 mL, 10.4 mmol) and (S) lactate ethyl ester (0.9 mL, 7.8 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM / EtOAc 3:1) to give **21** (370 mg, 30%) as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.33- 6.84 (m, 9H), 6.17-6.01 (m, 1H), 5.70 (m, 1H), 5.18-5.01 (m, 3H), 4.25-4.04 (m, 4H), 3.78-3.57 (m, 2H), 2.38-2.27 (m, 2H), 1.5-1.23 (m, 9H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 29.2 and 27.3; MS (ESI) 502 (M+Na).

#### Example 7D

Compound **22**: A solution of compound **21** (370mg) was dissolved in 8 mL of EtOH, then 0.12 mL of acetic acid and 10 % Pd/C (72 mg) was added. The resulted mixture was stirred under H<sub>2</sub> atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **22** (320mg, 96%) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) 7.27- 6.86 (m, 4H), 5.98 (s, 2H), 5.18-5.02 (m, 1H), 4.25-4.06 (m, 4H), 3.34-3.24 (m, 2H), 2.44-2.30 (m, 2H), 1.62-1.24 (m, 9H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 28.3 and 26.8; MS (ESI) 346 (M+1).

**Example 8A**

**Compound 24:** Compound 23 was purified using a Dynamax SD-200 HPLC system. The mobile phase consisted of acetonitrile: water (65:35, v/v) at a flow rate of 70 mL/ min. The injection volume was 4 mL. The detection was by fluorescence at 245 nm and peak area ratios were used for quantitations. Retention time was 8.2 min for compound 24 as yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.36-7.19 (m, 10H), 5.88 (m, 1H), 5.12 (s, 2H), 4.90-4.86 (m, 1H),

4.26-4.12 (m, 2H), 3.72-3.61(m, 2H), 2.36-2.29 (m, 2H), 1.79-1.74 (m, 2H); 1.27 (t, J = 7.2 Hz, 3H), 0.82 (t, J = 7.2 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  28.3; MS (ESI) 472 (M+Na).

#### Example 8B

Compound **25** was purified in the same manner and retention time was 7.9 min for compound **25** as yellow oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.34-7.14 (m, 10H), 5.75 (m, 1H), 5.10 (s, 2H), 4.96-4.91 (m, 1H), 4.18-4.12 (m, 2H), 3.66-3.55(m, 2H), 2.29-2.19 (m, 2H), 1.97-1.89 (m, 2H); 1.21 (t, J = 7.2 Hz, 3H), 0.97 (t, J = 7.2 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  26.2; MS (ESI) 472 (M+Na).

#### Example 8C

Compound **26**: A solution of compound **24** (1 g) was dissolved in 20 mL of EtOH, then 0.3 mL of acetic acid and 10 % Pd/C (200 mg) was added. The resulted mixture was stirred under  $\text{H}_2$  atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **26** (830mg, 99 %) as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.46-7.19 (m, 5H), 4.92-4.81 (m, 1H), 4.24-4.21 (m, 2H), 3.41-3.28 (m, 2H), 2.54-2.38 (m, 2H), 1.79-1.74 (m, 2H), 1.27 (t, J = 7.2 Hz, 3H), 0.80 (t, J = 7.2 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  26.9; MS (ESI) 316 (M+1).

#### Example 8D

Compound **27**: A solution of compound **25** (700g) was dissolved in 14 mL of EtOH, then 0.21 mL of acetic acid and 10 % Pd/C (140 mg) was added. The resulted mixture was stirred under  $\text{H}_2$  atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **27** (510mg, 98 %) as a colorless oil.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.39-7.18 (m, 5H), 4.98-4.85 (m, 1H), 4.25-4.22 (m, 2H), 3.43-3.28 (m, 2H), 2.59-2.41 (m, 2H), 1.99-1.85 (m, 2H), 1.28 (t, J = 7.2 Hz, 3H), 1.02 (t, J = 7.2 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  24.2; MS (ESI) 316 (M+1).

#### Example 8E

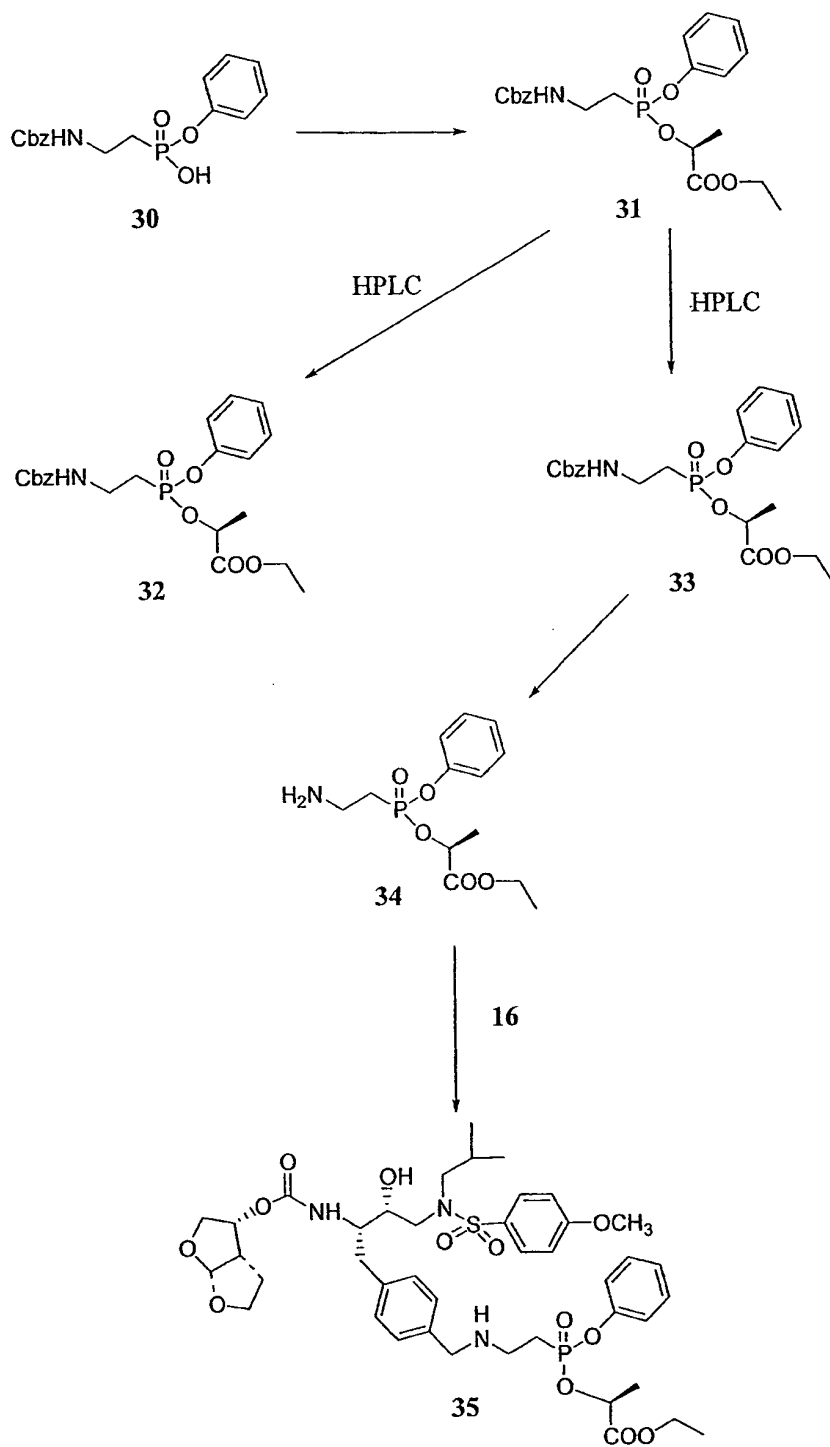
Compound **28**: To a stirred solution of compound **16** (1.18 g, 2 mmol) in 9 mL of 1,2-dichloroethane was added compound **26** (830 mg, 2.2 mmol) and  $\text{MgSO}_4$  (80 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (0.34



mL, 6 mmol) and sodium cyanoborohydride (251mg, 4 mmol) were added. The reaction mixture was stirred at room temperature for 2 h under argon. Then aqueous  $\text{NaHCO}_3$  (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **28** (880 mg, 50 %) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d,  $J$  = 8.7 Hz, 2H), 7.35-7.16 (m, 9H), 6.99 (d,  $J$  = 8.7 Hz, 2H), 5.64 (d,  $J$  = 5.4 Hz, 1H), 5.03-4.85 (m, 3H), 4.24 -3.67 (m overlapping s, 15H), 3.14-2.70 (m, 9H), 2.39-2.28 (m, 2H), 1.85-1.51 (m, 5H), 1.29-1.25 (m, 3H), 0.93-0.78 (m, 9H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  29.2; MS (ESI) 912 ( $\text{M}+\text{Na}$ ).

#### Example 8F

**Compound 29:** To a stirred solution of compound **16** (857 g, 1.45 mmol) in 7 mL of 1,2-dichloroethane was added compound **27** (600 mg, 1.6 mmol) and  $\text{MgSO}_4$  (60 mg), the resulted mixture was stirred at room temperature under argon for 3h, then acetic acid (0.23 mL, 3 mmol) and sodium cyanoborohydride (183mg, 2.9 mmol) were added. The reaction mixture was stirred at room temperature for 2 h under argon. Then aqueous  $\text{NaHCO}_3$  (50 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **29** (650 mg, 50 %) as a white solid.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.72 (d,  $J$  = 8.7 Hz, 2H), 7.35-7.16 (m, 9H), 7.00 (d,  $J$  = 8.7 Hz, 2H), 5.64 (d,  $J$  = 5.4 Hz, 1H), 5.03-4.90 (m, 3H), 4.17 -3.67 (m overlapping s, 15H), 3.16-2.77 (m, 9H), 2.26-2.19 (m, 2H), 1.94-1.53 (m, 5H), 1.26-1.18 (m, 3H), 1.00-0.87 (m, 9H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  27.4; MS (ESI) 912 ( $\text{M}+\text{Na}$ ).



Example 9A

Compound 31: To a stirred solution of compound 30 (20 g, 60 mmol) in 320 mL of toluene at room temperature under N<sub>2</sub> was added thionyl chloride (17.5 mL, 240 mmol) and a few drops of DMF. The resulted mixture was stirred at 60-70°C for 3 h. After cooled to room temperature, the solvent was removed under reduced pressure, and the residue was added 280 mL of DCM, followed by TEA (50 mL, 360 mmol) and (S) lactate ethyl ester (17 mL, 150 mmol). After 20h at room temperature, the solvent was removed under reduced pressure, and the residue was diluted with DCM and washed with brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (DCM / EtOAc, 1:1) to give 31 (24 g, 92 %) as a yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 28.2 and 26.2; MS (ESI) 458 (M+Na).

Example 9B

Compound 32: Compound 31 was purified using a Dynamax SD-200 HPLC system. The mobile phase consisted of acetonitrile: water (60:40, v/v) at a flow rate of 70 mL/ min. The injection volume was 3 mL. The detection was by fluorescence at 245 nm and peak area ratios were used for quantitations. Retention time was 8.1 min for compound 32 as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 28.2; MS (ESI) 458 (M+Na).

Example 9C

Compound 33 was purified in the same manner and retention time was 7.9 min for compound 33 as yellow oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.33-7.18 (m, 10H), 5.94-6.63 (m, 1H), 5.70 (m, 1H), 5.12-4.95 (m, 3H), 4.24-4.14 (m, 2H), 3.72-3.59(m, 2H), 2.35-2.20 (m, 2H), 1.58-1.19 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 26.2; MS (ESI) 458 (M+Na).

Example 9D

Compound 34: A solution of compound 33 (3.2 g) was dissolved in 60 mL of EtOH, then 0.9 mL of acetic acid and 10 % Pd/C (640 mg) was added. The resulted mixture was stirred

under H<sub>2</sub> atmosphere (balloon) for 4 h. After filtration through celite, the filtered was evaporated under reduced pressure to afford the compound **34** (2.7 g, 99 %) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.42-7.18 (m, 5H), 6.10 (s, 1H), 5.15-5.02 (m, 1H), 4.24-4.05 (m, 2H), 3.25-3.16 (m, 2H), 2.36-2.21 (m, 2H), 1.61-1.58 (m, 3H), 1.35- 1.18, m, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 26.1; MS (ESI) 302 (M+1).

#### Example 9E

Compound **35**: To a stirred solution of compound **16** (8.9 g, 15 mmol) in 70 mL of 1,2-dichloroethane was added compound **34** (8.3 g, 23 mmol) and MgSO<sub>4</sub> (80 mg), the resulted mixture was stirred at room temperature under argon for 2.5h, then acetic acid (3 mL, 52.5 mmol) and sodium cyanoborohydride (1.9g, 30 mmol) were added. The reaction mixture was stirred at room temperature for 1.5 h under argon. Then aqueous NaHCO<sub>3</sub> (100 mL) was added, and the mixture was extracted with ethyl acetate, and the combined organic layers were washed with brine and water, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The residue was purified by chromatography on silica gel (EtOH/EtOAc, 1/9) to give **35** (8.4 g, 64 %) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.73 (d, J = 8.7 Hz, 2H), 7.36-7.17(m, 9H), 7.00 (d, J = 8.7 Hz, 2H), 5.64 (d, J = 5.1 Hz, 1H), 5.07-4.97 (m, 3H), 4.19 -3.67 (m overlapping s, 13H), 3.15-2.78 (m, 9H), 2.25-2.19 (m, 2H), 1.91-1.54 (m, 6H), 1.24-1.20 (m, 3H), 0.94-0.87 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 27.4; MS (ESI) 876 (M+1).

#### Resolution of Compound 35 Diastereomers

Analysis was performed on an analytical Daicel Chiralcel OD column (fig. 3, 4), conditions described below, with a total of about 3.5 mg compound **35** free base injected onto the column. This lot was about a 3:1 mixture of major to minor diastereomers where the lactate ester carbon is a 3:1 mix of R and S configurations.

Two injections of 3.8 and 3.5 mg each were made using the conditions described below. The isolated major diastereomer fractions were evaporated to dryness on a rotary evaporator under house vacuum. The chromatographic solvents were displaced by two portions of ethyl acetate followed by a single portion of ethyl acetate – trifluoroacetic acid (about 95:5) and a final high vacuum strip to aid in removal of trace solvents. This yielded the major diastereomer trifluoroacetate salt as a gummy solid.

The resolved minor diastereomer was isolated for biological evaluation by an 11 mg injection, performed on an analytical Daicel Chiralcel OD column, using the conditions described in below. The minor diastereomer of 35 was isolated as the trifluoroacetate salt by the conditions described above.

Larger scale injections (~ 300 mg 35 per injection) were later performed on a Daicel Chiralcel OD column semi-preparative column with a guard column(fig. 5), conditions described below. A minimal quantity of isopropyl alcohol was added to heptane to dissolve the 3:1 diastereomeric mix of 35 and the resolved diastereomers sample, and the isolated fractions were refrigerated until the eluted mobile phase was stripped.

**Analytical Column, ~ 4 mg Injection, Heptane – EtOH (20:80) Initial (fig. 3)**

**HPLC CONDITIONS**

|                 |                                                                                                                               |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------|
| Column          | : Chiralcel OD, 10 $\mu$ m, 4.6 x 250 mm                                                                                      |
| Mobile Phase    | : Heptane – Ethyl Alcohol (20:80 initial)<br>: 100% Ethyl Alcohol (final)<br><b>Note: Final began after first peak eluted</b> |
| Flow Rate       | : 1.0 mL/min                                                                                                                  |
| Run Time        | : As needed                                                                                                                   |
| Detection       | : UV at 250 nm                                                                                                                |
| Temperature     | : Ambient                                                                                                                     |
| Injection       | : ~ 4 mg on Column                                                                                                            |
| Sample Prep.    | : Dissolved in ~ 1 mL heptane –<br>ethyl alcohol (50:50)                                                                      |
| Retention Times | : 35 Minor ~ 14 min<br>: 35 Major ~ 25 min                                                                                    |

**Analytical Column, ~ 6 mg Injection, Heptane – EtOH (65:35) Initial (fig. 4)**

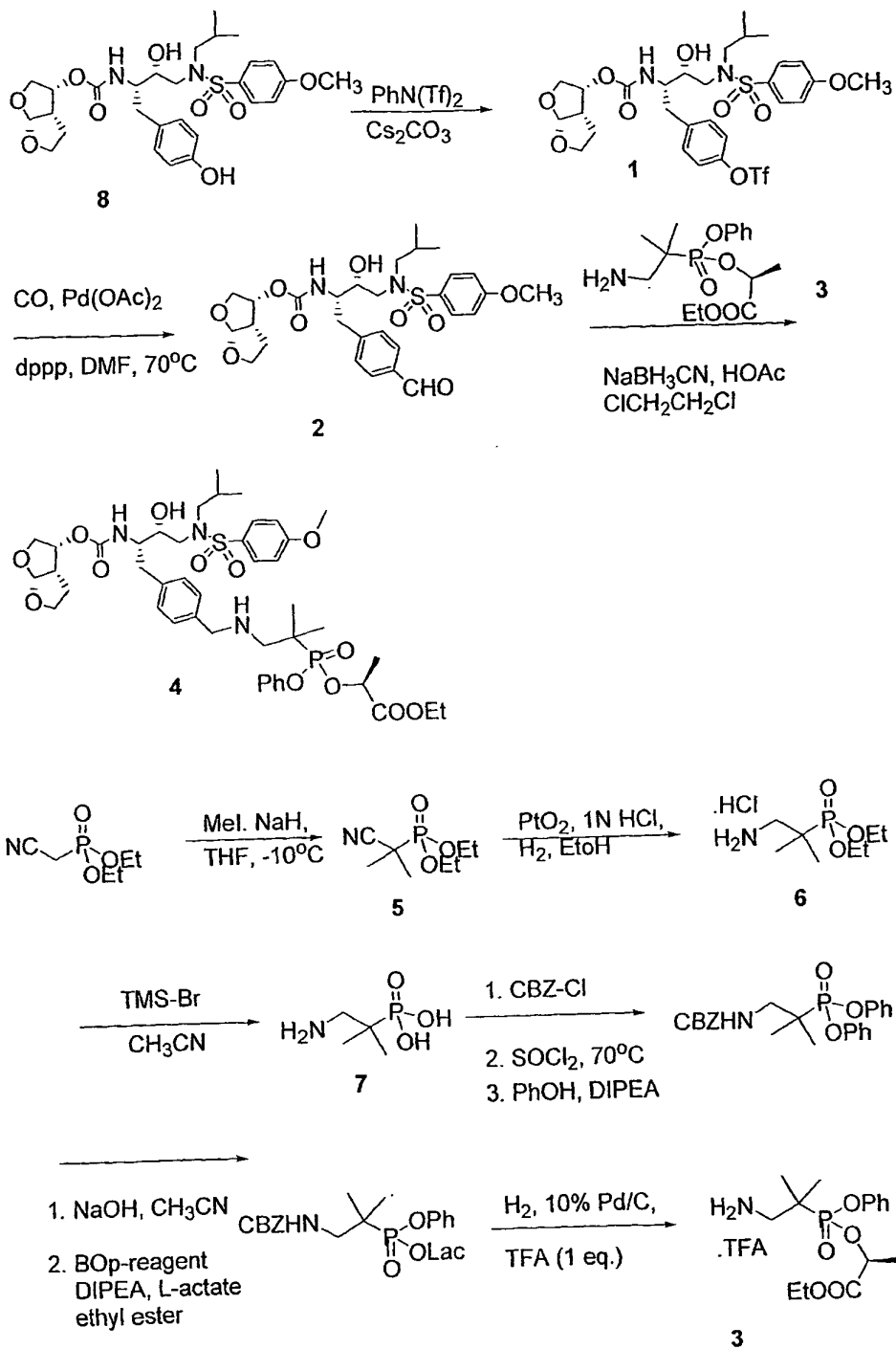
**HPLC CONDITIONS**

|                 |                                                                                                                                                                                                                                                                         |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Column          | : Chiralcel OD, 10 $\mu$ m, 4.6 x 250 mm                                                                                                                                                                                                                                |
| Mobile Phase    | : Heptane – Ethyl Alcohol (65:35 initial)<br>: Heptane – Ethyl Alcohol (57.5:42.5 intermediate)<br>Note: Intermediate began after impurity peaks eluted<br>: Heptane – Ethyl Alcohol (20:80 final)<br>Note: Final mobile phase began after minor<br>diastereomer eluted |
| Flow Rate       | : 1.0 mL/min                                                                                                                                                                                                                                                            |
| Run Time        | : As needed                                                                                                                                                                                                                                                             |
| Detection       | : UV at 250 nm                                                                                                                                                                                                                                                          |
| Temperature     | : Ambient                                                                                                                                                                                                                                                               |
| Injection       | : ~ 4 mg on Column                                                                                                                                                                                                                                                      |
| Sample Prep.    | : Dissolved in ~ 1 mL heptane –<br>ethyl alcohol (50:50)                                                                                                                                                                                                                |
| Retention Times | : 35 Minor ~ 14 min<br>: 35 Major ~ 40 min                                                                                                                                                                                                                              |

**Semi-Preparative Column, ~ 300 mg Injection, Heptane – EtOH (65:35) Initial (fig. 5)**

**HPLC CONDITIONS**

|                 |                                                                           |
|-----------------|---------------------------------------------------------------------------|
| Columns         | : Chiralcel OD, 20 $\mu$ m, 21 x 50 mm (guard)                            |
|                 | : Chiralcel OD, 20 $\mu$ m, 21 x 250 mm                                   |
| Mobile Phase    | : Heptane – Ethyl Alcohol (65:35 initial)                                 |
|                 | : Heptane – Ethyl Alcohol (50:50 intermediate)                            |
|                 | Note: Intermediate began after minor<br>diastereomer peak eluted          |
|                 | : Heptane – Ethyl Alcohol (20:80 final)                                   |
|                 | Note: Final mobile phase began after major<br>diastereomer began to elute |
| Flow Rate       | : 10.0 mL/min                                                             |
| Run Time        | : As needed                                                               |
| Detection       | : UV at 260 nm                                                            |
| Temperature     | : Ambient                                                                 |
| Injection       | : ~ 300 mg on Column                                                      |
| Sample Prep.    | : Dissolved in ~ 3.5 mL heptane –<br>ethyl alcohol (70:30)                |
| Retention Times | : 35 Minor ~ 14 min                                                       |
|                 | : 35 Major ~ 40 min                                                       |





Example 29

Triflate derivative 1: A THF-CH<sub>2</sub>Cl<sub>2</sub> solution (30mL-10 mL) of 8 (4 g, 6.9 mmol), cesium carbonate (2.7 g, 8 mmol), and N-phenyltrifluoromethane sulfonimide (2.8 g, 8 mmol) was reacted overnight. The reaction mixture was worked up, and concentrated to dryness to give crude triflate derivative 1.

Aldehyde 2: Crude triflate 1 (4.5 g, 6.9 mmol) was dissolved in DMF (20 mL), and the solution was degassed (high vacuum for 2 min, Ar purge, repeat 3 times). Pd(OAc)<sub>2</sub> (0.12 g, 0.27 mmol), and bis(diphenylphosphino)propane (dppp, 0.22 g, 0.27 mmol) were added, the solution was heated to 70°C. Carbon monoxide was rapidly bubbled through the solution, then under 1 atmosphere of carbon monoxide. To this solution were slowly added TEA (5.4 mL, 38 mmol), and triethylsilane (3 ml), 18 mmol). The resulting solution was stirred overnight at room temperature. The reaction mixture was worked up, and purified on silica gel column chromatograph to afford aldehyde 2 (2.1 g, 51 %). (Hostetler, et al J. Org. Chem., 1999. 64, 178-185).

Lactate prodrug 4: Compound 4 is prepared as described above procedure for Example 9E, Compound 35 by the reductive amination between 2 and 3 with NaBH<sub>3</sub>CN in 1,2-dichloroethane in the presence of HOAc.

Example 30 Preparation of Compound 3

Diethyl (cyano(dimethyl)methyl) phosphonate 5: A THF solution (30 mL) of NaH (3.4 g of 60% oil dispersion, 85 mmol) was cooled to -10°C, followed by the addition of diethyl (cyanomethyl)phosphonate (5g, 28.2 mmol) and iodomethane (17 g, 112 mmol). The resulting solution was stirred at -10°C for 2 hr, then 0°C for 1 hr, was worked up, and purified to give dimethyl derivative 5 (5 g, 86 %).

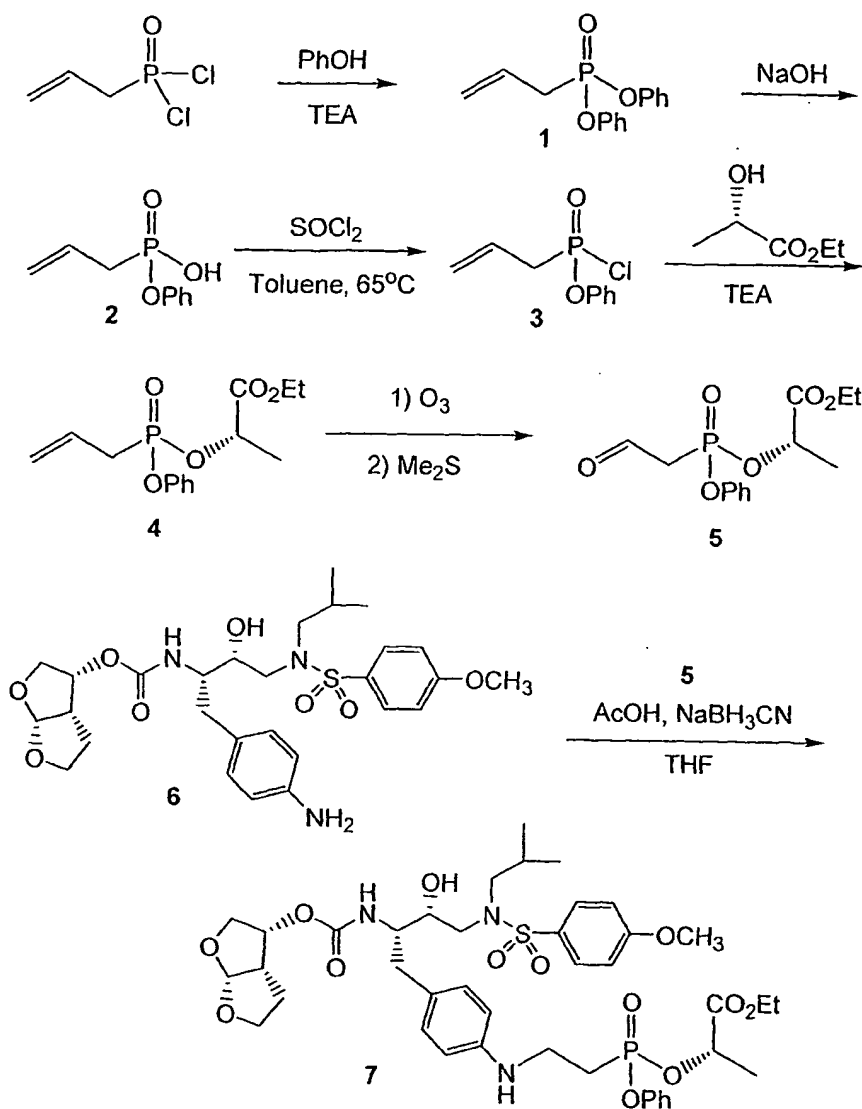
Diethyl (2-amino-1,1-dimethyl-ethyl)phosphonate 6: Compound 5 was reduced to amine derivative 6 by the described procedure (J. Med. Chem. 1999, 42, 5010-5019).

A solution of ethanol (150 mL) and 1N HCl aqueous solution (22 mL) of 5 (2.2 g, 10.7 mmol) was hydrogenated at 1 atmosphere in the presence of PtO<sub>2</sub> (1.25 g) at room temperature overnight. The catalyst was filtered through a celite pad. The filtrate was concentrated to dryness, to give crude 6 (2.5g, as HCl salt).

2-Amino-1,1-dimethyl-ethyl phosphonic acid 7: A solution of  $\text{CH}_3\text{CN}$  (30 mL) of crude 6 (2.5 g) was cooled to  $0^\circ\text{C}$ , and treated with  $\text{TMSBr}$  (8 g, 52 mmol) for 5 hr. The reaction mixture was stirred with methanol for 1.5 hr at room temperature, concentrated, recharged with methanol, concentrated to dryness to give crude 7 which was used for next reaction without further purification.

Lactate phenyl (2-amino-1,1-dimethyl-ethyl)phosphonate 3: Compound 3 is synthesized according to the procedures described in Example 9D, Compound 34 for the preparation of lactate phenyl 2-aminoethyl phosphonate 34. Compound 7 is protected with CBZ, followed by the reaction with thionyl chloride at  $70^\circ\text{C}$ . The CBZ protected dichlorodate is reacted phenol in the presence of DIPEA. Removal of one phenol, follow by coupling with ethyl L-lactate leads N-CBZ-2-amino-1,1-dimethyl-ethyl phosphonate derivative. Hydrogenation of N-CBZ derivative at 1 atmosphere in the presence of 10 % Pd/C and 1 eq. of TFA affords compound 3 as TFA salt.

Scheme 1

**Example 1**

**Monophenol Allylphosphonate 2:** To a solution of allylphosphonic dichloride (4 g, 25.4 mmol) and phenol (5.2 g, 55.3 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL) at 0°C was added TEA (8.4 mL, 60 mmol). After stirred at room temperature for 1.5 h, the mixture was diluted with hexane-ethyl acetate and washed with HCl (0.3 N) and water. The organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was filtered through a pad of silica gel (eluted with 2:1 hexane-ethyl acetate) to afford crude product diphenol

allylphosphonate **1** (7.8 g, containing the excessive phenol) as an oil which was used directly without any further purification. The crude material was dissolved in CH<sub>3</sub>CN (60 mL), and NaOH (4.4N, 15 mL) was added at 0°C. The resulted mixture was stirred at room temperature for 3 h, then neutralized with acetic acid to pH = 8 and concentrated under reduced pressure to remove most of the acetonitrile. The residue was dissolved in water (50 mL) and washed with CH<sub>2</sub>Cl<sub>2</sub> (3X25 mL). The aqueous phase was acidified with concentrated HCl at 0°C and extracted with ethyl acetate. The organic phase was dried over MgSO<sub>4</sub>, filtered, evaporated and co-evaporated with toluene under reduced pressure to yield desired monophenol allylphosphonate **2** (4.75 g, 95%) as an oil.

#### Example 2

**Monolactate Allylphosphonate 4:** To a solution of monophenol allylphosphonate **2** (4.75 g, 24 mmol) in toluene (30 mL) was added SOCl<sub>2</sub> (5 mL, 68 mmol) and DMF (0.05 mL). After stirred at 65°C for 4 h, the reaction was completed as shown by <sup>31</sup>P NMR. The reaction mixture was evaporated and co-evaporated with toluene under reduced pressure to give monochloride **3** (5.5 g) as an oil. To a solution of chloride **3** in CH<sub>2</sub>Cl<sub>2</sub> (25 mL) at 0°C was added ethyl (s)-lactate (3.3 mL, 28.8 mmol), followed by TEA. The mixture was stirred at 0°C for 5 min then at room temperature for 1 h, and concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N), the organic phase was washed with water, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired monolactate **4** (5.75 g, 80%) as an oil (2:1 mixture of two isomers): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.1-7.4 (m, 5H), 5.9 (m, 1H), 5.3 (m, 2H), 5.0 (m, 1H), 4.2 (m, 2H), 2.9 (m, 2H), 1.6; 1.4 (d, 3H), 1.25 (m, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 25.4, 23.9.

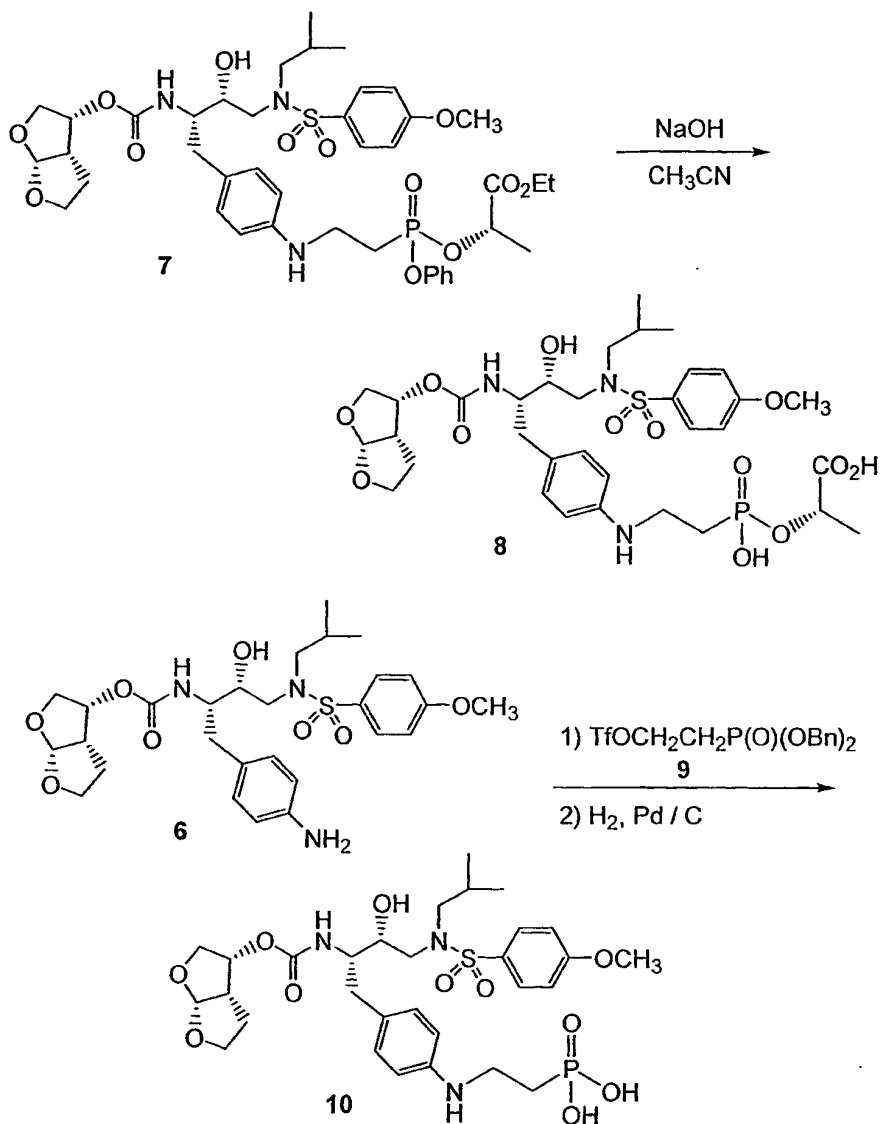
#### Example 3

**Aldehyde 5:** A solution of allylphosphonate **4** (2.5 g, 8.38 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was bubbled with ozone air at -78°C until the solution became blue, then bubbled with nitrogen until the blue color disappeared. Methyl sulfide (3 mL) was added at -78°C. The mixture was warmed up to room temperature, stirred for 16 h and concentrated under reduced pressure to give desired aldehyde **5** (3.2 g, as a 1:1 mixture of DMSO): <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 9.8 (m, 1H), 7.1-7.4 (m, 5H), 5.0 (m, 1H), 4.2 (m, 2H), 3.4 (m, 2H), 1.6; 1.4 (d, 3H), 1.25 (m, 3H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 17.7, 15.4.

Example 4

Compound 7: To a solution of aniline 6 (reported before) (1.62 g, 2.81 mmol) in THF (40 mL) was added acetic acid (0.8 mL, 14 mmol), followed by aldehyde 5 (1.3 g, 80%, 3.46 mmol) and  $\text{MgSO}_4$  (3 g). The mixture was stirred at room temperature for 0.5 h, then  $\text{NaBH}_3\text{CN}$  (0.4 g, 6.37 mmol) was added. After stirred for 1 h, the reaction mixture was filtered. The filtrate was diluted with ethyl acetate and washed with  $\text{NaHCO}_3$ , dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to give compound 6 (1.1g, 45%) as a 3:2 mixture of two isomers, which were separated by HPLC (mobile phase, 70%  $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ ; flow rate: 70 mL/min; detection: 254 nm; column:  $8\mu\text{ C18}$ , 41X250 mm, Varian). Isomer A (0.39 g):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75 (d, 2H), 7.1-7.4 (m, 5H), 7.0 (m, 4H), 6.6 (d, 2H), 5.65 (d, 1H), 5.05 (m, 2H), 4.9 (d, 1H), 4.3 (brs, 1H), 4.2 (q, 2H), 3.5-4.0 (m, 6H), 3.9 (s, 3H), 2.6-3.2 (m, 9H), 2.3 (m, 2), 1.6-1.9 (m, 5H), 1.25 (t, 3H), 0.9 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  26.5; MS (ESI): 862 (M+H). Isomer B (0.59 g):  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75 (d, 2H), 7.1-7.4 (m, 5H), 7.0 (m, 4H), 6.6 (d, 2H), 5.65 (d, 1H), 5.05 (m, 2H), 4.9 (d, 1H), 4.5 (brs, 1H), 4.2 (q, 2H), 3.5-4.0 (m, 6H), 3.9 (s, 3H), 2.7-3.2 (m, 9H), 2.4 (m, 2), 1.6-1.9 (m, 2H), 1.4 (d, 3H), 1.25 (t, 3H), 0.9 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  28.4; MS (ESI): 862 (M+H).

Scheme 2

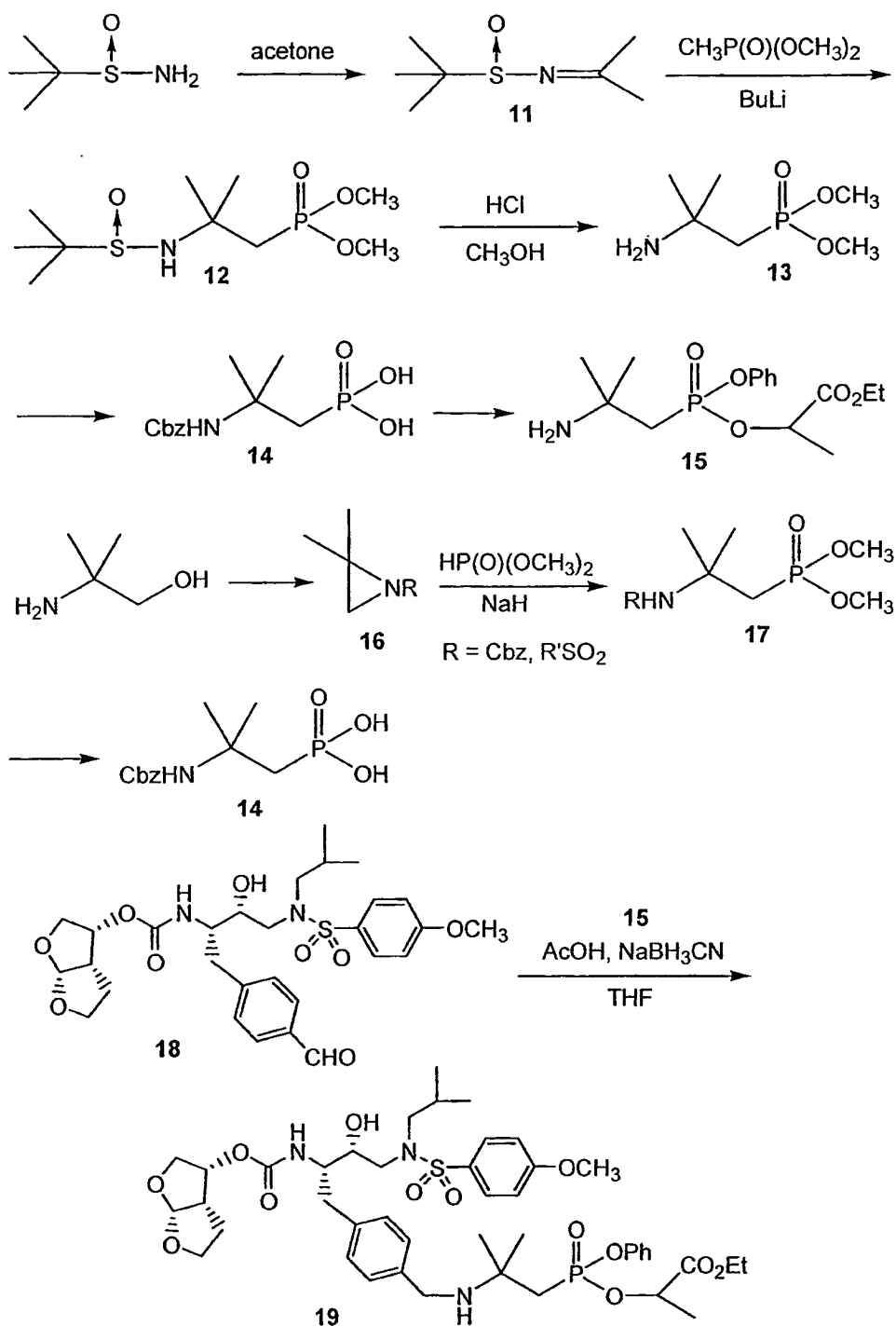
**Example 5**

**Acid 8:** To a solution of compound 7 (25 mg, 0.029 mmol) in acetonitrile (1 mL) at 0°C was added NaOH (1N, 0.125 mL). The mixture was stirred at 0°C for 0.5 h and at room temperature for 1 h. The reaction was quenched with acetic acid and purified by HPLC to give acid 8 (10 mg, 45%). <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.8 (d, 2H), 7.5 (d, 2H), 7.4 (d, 2H), 7.1 (d, 2H), 5.6 (d, 1H), 4.9 (m, 3H), 3.2-4.0 (m, 6H), 3.9 (s, 3H), 2.6-3.2 (m, 9H), 2.05 (m, 2), 1.4-1.7 (m, 2H), 1.5 (d, 3H), 0.9 (2d, 6H); <sup>31</sup>P NMR (CD<sub>3</sub>OD) δ 20.6; MS (ESI): 758 (M+H).

Example 6

**Diacid 10:** To a solution of triflate **9** (94 mg, 0.214 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added a solution of aniline **6** (100 mg, 0.173 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) at  $-40^\circ\text{C}$ , followed by 2,6-lutidine (0.026 mL). The mixture was warmed up to room temperature and stirred for 1 h. Cesium carbonate (60 mg) was added and the reaction mixture was stirred for additional 1 h. The mixture was diluted with ethyl acetate, washed with HCl (0.2N), dried over  $\text{MgSO}_4$ , filtered and concentrated under reduced pressure. The residue was purified by HPLC to afford dibenzyl phosphonate (40 mg). To a solution of this dibenzyl phosphonate in ethanol (3 mL) and ethyl acetate (1 mL) was added 10% Pd/C (40 mg). The mixture was stirred under hydrogen atmosphere (balloon) for 4 h. The reaction mixture was diluted with methanol, filtered and concentrated under reduced pressure. The residue was washed with ethyl acetate and dried to give desired product diacid **10** (20 mg).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  7.8 (d, 2H), 7.3 (d, 2H), 7.1 (2d, 4H), 5.6 (d, 1H), 4.9 (m, 2H), 3.4-4.0 (m, 6H), 3.9 (s, 3H), 2.5-3.2 (m, 9H), 2.0 (m, 2), 1.4-1.7 (m, 2H), 0.9 (2d, 6H);  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  22.1; MS (ESI): 686 (M+H).

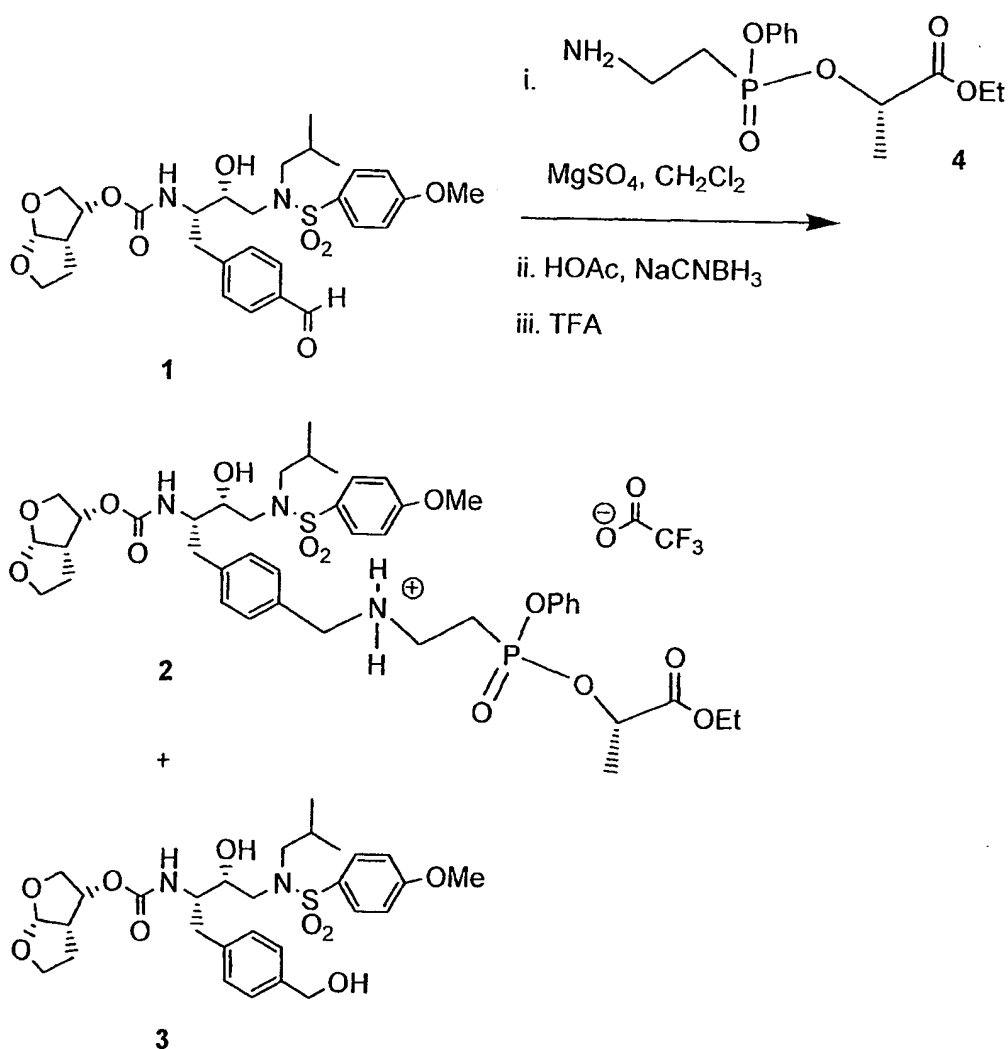
Scheme 3



The synthesis of compound **19** is outlined in Scheme 3. Condensation of 2-methyl-2-propanesulfinamide with acetone give sulfinyl imine **11** (J. Org. Chem. 1999, 64, 12).



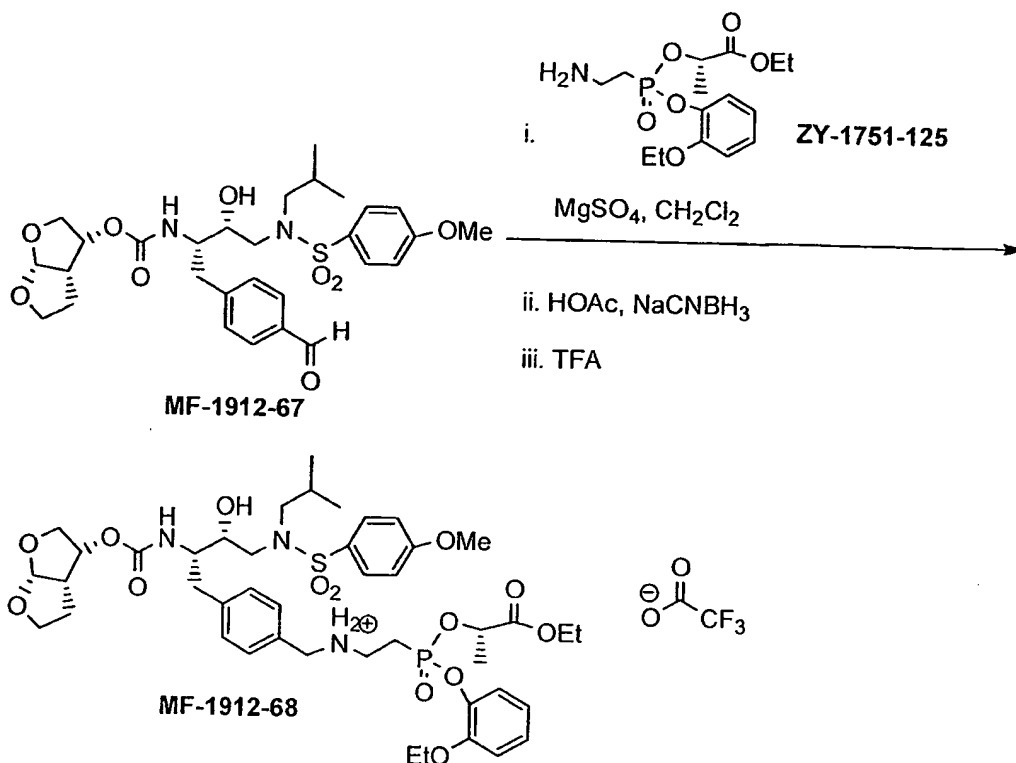
Addition of dimethyl methylphosphonate lithium to **11** afford **12**. Acidic methanolysis of **12** provide amine **13**. Protection of amine with Cbz group and removal of methyl groups yield phosphonic acid **14**, which can be converted to desired **15** using methods reported earlier on. An alternative synthesis of compound **14** is also shown in Scheme 3. Commercially available 2-amino-2-methyl-1-propanol is converted to aziridines **16** according to literature methods (J. Org. Chem. 1992, 57, 5813; and Syn. Lett. 1997, 8, 893). Aziridine opening with phosphite give **17** (Tetrahedron Lett. 1980, 21, 1623). Deprotection (and, if necessary, reprotection) of **17** afford **14**. Reductive amination of amine **15** and aldehyde **18** provides compound **19**.



Example 1

2-{{[2-(4-{2-(Hexahydro-furo[2,3-b]furan-3-yloxy-carbonylamino)-3-hydroxy-4-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-butyl}-benzylamino)-ethyl]-phenoxy-phosphinoyloxy}-propionic acid ethyl ester **2** (Compound 35, previous Example 9E).

A solution of **1** (2.07 g, 3.51 mmol) and **4** (1.33 g, 3.68 mmol) of a 4:1 mixture of two diastereomers at the phosphorous center) were dissolved in 14 mL of (CH<sub>2</sub>Cl<sub>2</sub>)<sub>2</sub> to provide a clear solution. Addition of MgSO<sub>4</sub> (100 mg) to the solution resulted in a white cloudy mixture. The solution was stirred at ambient temperature for 3 hours when acetic acid (0.80 mL, 14.0 mmol) and sodium cyanoborohydride (441 mg, 7.01 mmol) were added. Following the reaction progress by TLC showed complete consumption of the aldehyde starting materials in 1 hour. The reaction mixture was worked up by addition of 200 mL of saturated aqueous NaHCO<sub>3</sub> and 400 mL of CH<sub>2</sub>Cl<sub>2</sub>. The aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub> two more times (2 x 300 mL). The combined organic extracts were dried *in vacuo* and purified by column chromatography (EtOAc- 10% MeOH: EtOAc) to provide the desired product as a foam. The early eluting compound from the column was collected and characterized as alcohol **3** (810 mg, 39%). Addition of TFA (3 x 1 mL) generated the TFA salt which was lyophilized from 50 mL of a 1:1 CH<sub>3</sub>CN: H<sub>2</sub>O to provide 1.63 g (47%) of the product **2** as a white powder. <sup>1</sup>H NMR (CD<sub>3</sub>CN) δ 8.23 (br s, 2H), 7.79 (d, *J*= 8.4 Hz, 2H), 7.45- 7.13 (m, 9H), 7.09 (d, *J*= 8.4 Hz, 2H), 5.86 (d, *J*= 9.0 Hz, 1H), 5.55 (d, *J*= 4.8 Hz, 1H), 5.05-4.96 (m, 1H), 4.96- 4.88 (m, 1H), 4.30-4.15 (m, 4H), 3.89 (s, 3H), 3.86- 3.76 (m, 4H), 3.70- 3.59 (m, 4H), 3.56- 3.40 (m, 2H), 3.34 (d, *J*= 15 Hz, 1H), 3.13 (d, *J*= 13.5 Hz, 1H), 3.06- 2.93 (m, 2H), 2.92- 2.80 (m, 2H), 2.69- 2.43 (m, 3H), 2.03- 1.86 (m, 1H), 1.64- 1.48 (m, 1H), 1.53 and 1.40 (d, *J*= 6.3 Hz, *J*= 6.6 Hz, 3H), 1.45- 1.35 (m, 1H), 1.27 and 1.23 (t, *J*= 6.9 Hz, *J*= 7.2 Hz, 3H), 0.90 (t, *J*= 6.9 Hz, 6H). <sup>31</sup>P NMR (CD<sub>3</sub>CN) δ 24.47, 22.86. ESI (M+ H)<sup>+</sup> 876.4.

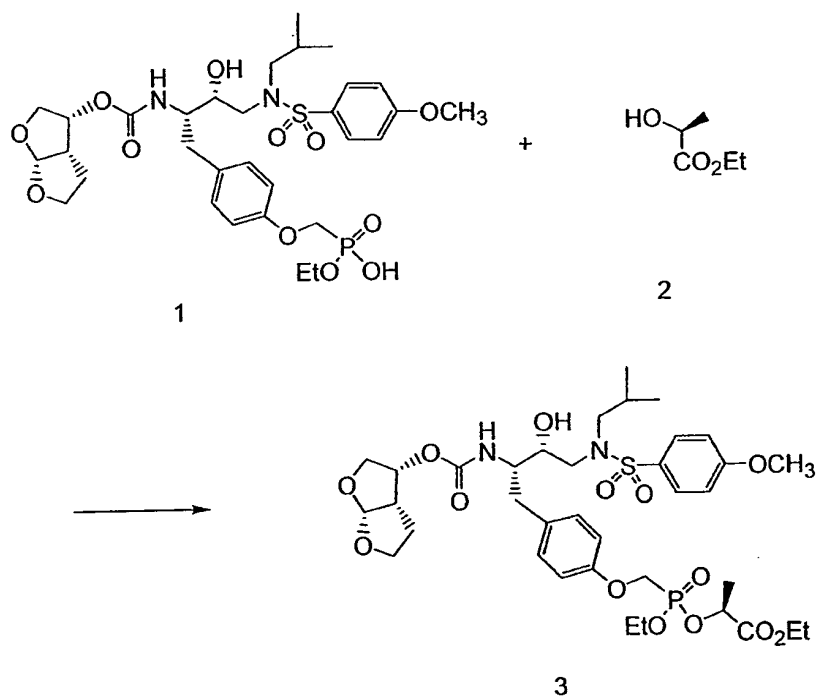
Example 2

2-[[2-(4-{2-(Hexahydro-furo[2,3-b]furan-3-yloxycarbonylamino)-3-hydroxy-4-[isobutyl-(4-methoxy-benzenesulfonyl)-amino]-butyl}-benzylamino)-ethyl]-phenoxy-phosphinoyloxy]-propionic acid ethyl ester (**MF-1912-68**):

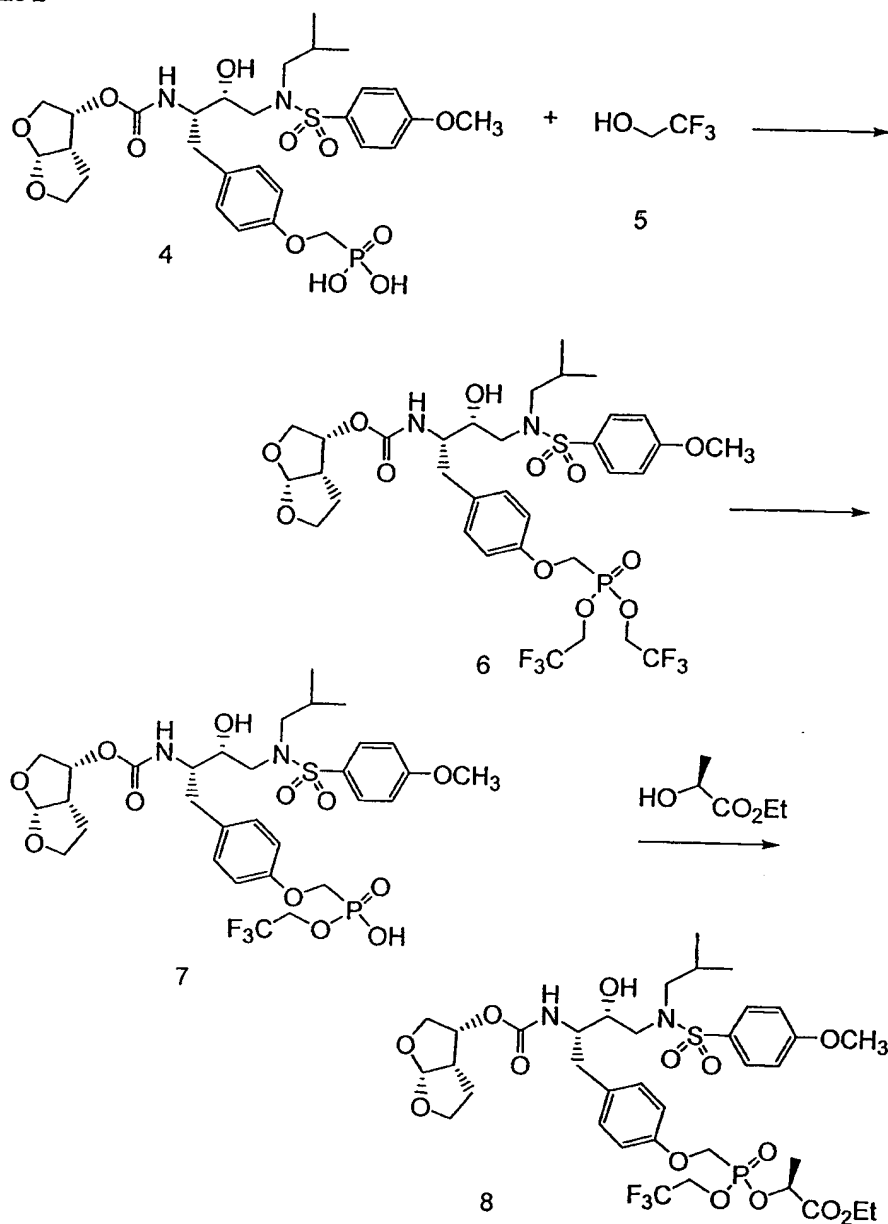
A solution of **MF-1912-67** (0.466 g, 0.789 mmol) and **ZY-1751-125** (0.320 g, 0.789 mmol of a 1:1 mixture of two diastereomers at the phosphorous center) were dissolved in 3.1 mL of  $(\text{CH}_2\text{Cl}_2)_2$  to provide a clear solution. Addition of  $\text{MgSO}_4$  (20 mg) to the solution resulted in a white cloudy mixture. The solution was stirred at ambient temperature for 3 hours when acetic acid (0.181 mL, 3.16 mmol) and sodium cyanoborohydride (99 mg, 1.58 mmol) were added. Following the reaction progress by TLC showed complete consumption of the aldehyde starting materials in 1.5 hour. The reaction mixture was worked up by addition of 50 mL of saturated aqueous  $\text{NaHCO}_3$  and 200 mL of  $\text{CH}_2\text{Cl}_2$ . The aqueous layer was extracted with  $\text{CH}_2\text{Cl}_2$  two more times (2 x 200 mL). The combined organic extracts were dried *in vacuo* and purified by column chromatography (EtOAc- 10% MeOH: EtOAc) to provide the desired product as a foam. The early eluting compound from the column was collected and characterized to be MF-1912-48b alcohol (190 mg, 41%). Addition of TFA (3 x 1 mL) generated the TFA salt which was lyophilized from 50 mL of a 1:1  $\text{CH}_3\text{CN}$ :  $\text{H}_2\text{O}$  to

provide 0.389 g (48%) of the product as a white powder.  $^1\text{H}$  NMR ( $\text{CD}_3\text{CN}$ )  $\delta$  8.39 (br s, 2H), 7.79 (d,  $J=8.7$  Hz, 2H), 7.40 (d,  $J=7.5$  Hz, 2H), 7.34 (d,  $J=8.1$  Hz, 2H), 7.26-7.16 (m, 2H), 7.10 (d,  $J=9$  Hz, 3H), 7.01-6.92 (m, 1H), 5.78 (d,  $J=9.0$  Hz, 1H), 5.55 (d,  $J=5.1$  Hz, 1H), 5.25-5.03 (m, 1H), 4.95-4.88 (m, 1H), 4.30-4.17 (m, 4H), 4.16-4.07 (m, 2H), 3.90 (s, 3H), 3.88-3.73 (m, 4H), 3.72-3.60 (m, 2H), 3.57-3.38 (m, 2H), 3.32 (br d,  $J=15.3$  Hz, 1H), 3.13 (br d,  $J=14.7$  Hz, 1H), 3.05-2.92 (m, 2H), 2.92-2.78 (m, 2H), 2.68-2.48 (m, 3H), 2.03-1.90 (m, 1H), 1.62-1.51 (m, 1H), 1.57 and 1.46 (d,  $J=6.9$  Hz,  $J=6.9$  Hz, 3H), 1.36-1.50 (m, 1H), 1.43-1.35 (m, 4H), 1.33-1.22 (m, 3H), 0.91 (t,  $J=6.6$  Hz, 6H).  $^{31}\text{P}$  NMR ( $\text{CD}_3\text{CN}$ )  $\delta$  25.27, 23.56. ESI ( $\text{M}+\text{H}$ ) $^+$  920.5.

Scheme 1



Scheme 2

**Example 1**

**Mono-Ethyl mono-lactate 3:** To a solution of 1 (96mg, 0.137 mmol) and ethyl lactate 2 (0.31 mL, 2.7 mmol) in pyridine (2 mL) was added N, N-dicyclohexylcarbodiimide (170 mg, 0.822 mmol). The solution was stirred for 18h at 70°C. The mixture was cooled to room temperature and diluted with dichloromethane. The solid was removed by filtration and the filtrate was concentrated. The residue was suspended in diethyl ether/dichloromethane and

filtered again. The filtrate was concentrated and mixture was chromatographed on silica gel eluting with EtOAc/hexane to provide compound 3 (43 mg, 40%) as a foam:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.00 (d, 2H); 7.00 (d, 2H), 6.88 (d, 2H), 5.67 (d, 1H), 4.93-5.07 (m, 2H), 4.15-4.39 (m, 6H), 3.70-3.99 (m, 10H), 2.76-3.13 (m, 7H), 1.55-1.85 (m, 9H), 1.23-1.41 (m, 6H), 0.90 (dd, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.1, 20.2; MS (ESI) 823 (M+Na).

#### Example 2

Bis-2,2,2-trifluoroethyl phosphonate 6: To a solution of 4 (154mg, 0.228 mmol) and 222,-trifluoroethanol 5 (1 mL, 13.7 mmol) in pyridine (3 mL) was added N, N-dicyclohexylcarbodiimide (283 mg, 1.37 mmol). The solution was stirred for 6.5h at 70°C. The mixture was cooled to room temperature and diluted with dichloromethane. The solid was removed by filtration and the filtrate was concentrated. The residue was suspended in dichloromethane and filtered again. The filtrate was concentrated and mixture was chromatographed on silica gel eluting with EtOAc/hexane to provide compound 6 (133 mg, 70%) as a foam:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.21 (d, 2H); 7.00 (d, 2H), 6.88 (dd, 2H), 5.66 (d, 1H), 4.94-5.10 (m, 3H), 4.39-4.56 (m, 6H), 3.71-4.00 (m, 10H), 2.77-3.18 (m, 7H), 1.67-1.83(m, 2H), 0.91 (dd, 4H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  22.2; MS (ESI) 859 (M+Na).

#### Example 3

Mono-2,2,2-trifluoroethyl phosphonate 7: To a solution of 6 (930mg, 1.11 mmol) in THF (14 mL) and water (10 mL) was added an aqueous solution of NaOH in water (1N, 2.2 mL). The solution was stirred for 1h at 0°C. An excess amount of Dowex resin ( $\text{H}^+$ ) was added to until pH=1. The mixture was filtered and the filtrate was concentrated under reduced pressure. The concentrated solution was azeotroped with EtOAc/toluene three times and the white powder was dried *in vacuo* provide compound 7 (830 mg, 100%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.11 (d, 2H); 6.99 (d, 2H), 6.85 (d, 2H), 5.63 (d, 1H), 5.26 (m, 1H), 5.02 (m, 1H), 4.40 (m, 1H), 4.14 (m, 4H), 3.60-3.95 (m, 12H), 2.62-3.15 (m, 15H), 1.45-1.84 (m, 3H), 1.29 (m, 4H), 0.89 (d, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.9; MS (ESI) 723 (M+Na).

#### Example 4

Mono-2,2,2-trifluoroethyl mono-lactate 8: To a solution of 7 (754mg, 1 mmol) and N, N-dicyclohexylcarbodiimide (1.237 g, 6 mmol) in pyridine (10 mL) was added ethyl lactate

(2.26 mL, 20 mmol). The solution was stirred for 4.5h at 70°C. The mixture was concentrated and the residue was suspended in diethyl ether (5 mL) and dichloromethane (5 mL) and filtered. The solid was washed a few times with diethyl ether. The combined filtrate was concentrated and the crude product was chromatographed on silica gel, eluting with EtOAc and hexane to provide compound 8 (610 mg, 71%) as a foam.  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, 2H), 7.16 (d, 2H); 6.99 (d, 2H), 6.88 (dd, 2H), 5.66 (d, 1H), 4.95-5.09 (m, 2H), 4.19-4.65 (m, 6H), 3.71-4.00 (m, 9H), 2.76-3.13 (m, 6H), 1.57-1.85 (m, 7H), 1.24-1.34 (m, 4H), 0.91 (dd, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.29, 21.58; MS (ESI) 855 (M+1).

#### Example 1

**Boc-protected hydroxylamine 1:** A solution of diethyl hydroxymethyl phosphonate triflate (0.582 g, 1.94 mmol) in dichloromethane (19.4 mL) was treated with triethylamine (0.541 mL, 3.88 mmol). Tert-butyl N-hydroxy-carbamate (0.284 g, 2.13 mmol) was added and the reaction mixture was stirred at room temperature overnight. The mixture was partitioned between dichloromethane and water. The organic phase was washed with saturated NaCl, dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (1/1 – ethyl acetate/hexane) affording the BOC-protected hydroxylamine 1 (0.41 g, 75%) as an oil:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.83 (s, 1H), 4.21 (d, 2H), 4.18 (q, 4H), 1.47 (s, 9H), 1.36 (t, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  19.3.

#### Example 2

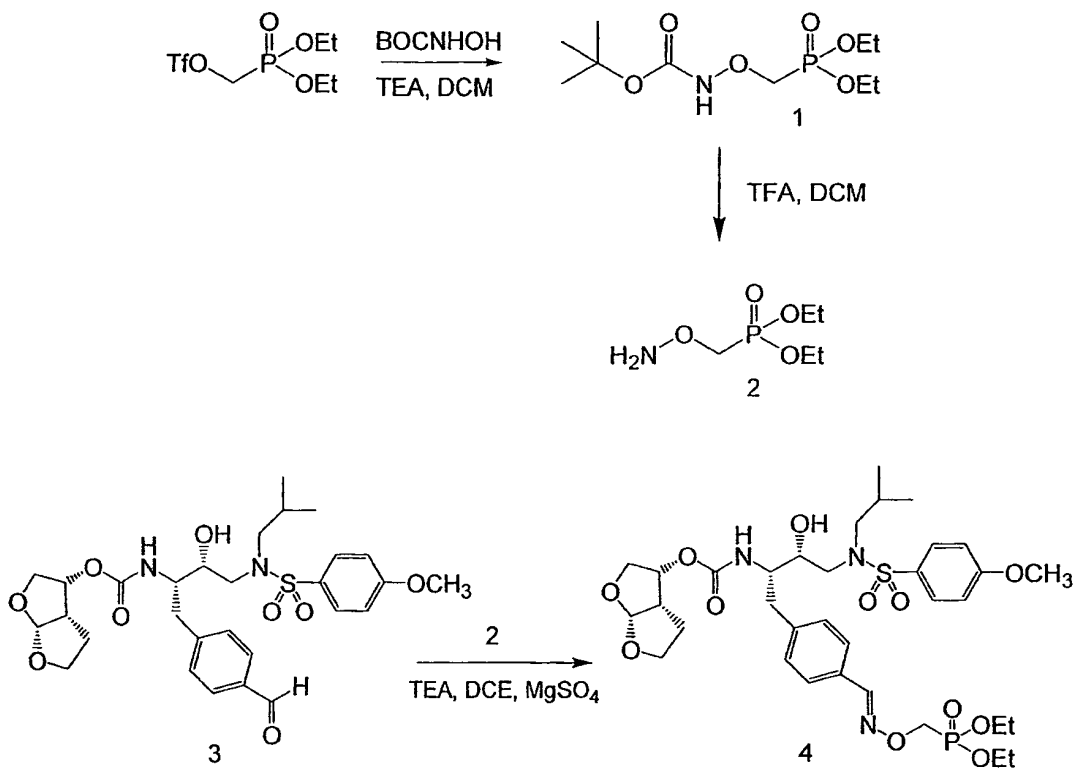
**Hydroxylamine 2:** A solution of BOC-protected hydroxylamine 1 (0.305 g, 1.08 mmol) in dichloromethane (2.40 mL) was treated with trifluoroacetic acid (0.829 mL, 10.8 mmol). The reaction was stirred for 1.5 hours at room temperature and then the volatiles were evaporated under reduced pressure with toluene to afford the hydroxylamine 2 (0.318 g, 100%) as the TFA salt which was used directly without any further purification:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  10.87 (s, 2H), 4.45 (d, 2H), 4.24 (q, 4H), 1.38 (t, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.9; MS (ESI) 184 (M+H).

#### Example 3

**Oxime 4:** To a solution of aldehyde 3 (96 mg, 0.163 mmol) in 1,2-dichloroethane (0.65 mL) was added hydroxylamine 2 (72.5 mg, 0.244 mmol), triethylamine (22.7  $\mu\text{L}$ , 0.163 mmol) and  $\text{MgSO}_4$  (10 mg). The reaction mixture was stirred at room temperature for 2 hours then

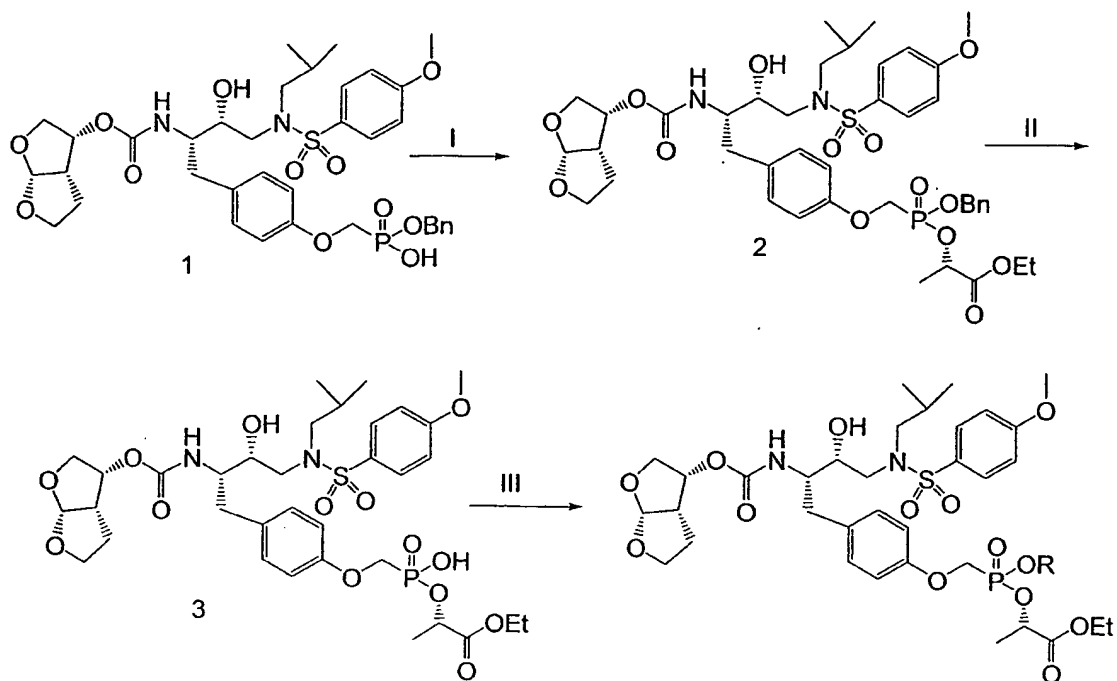
the mixture was partitioned between dichloromethane and water. The organic phase was washed with saturated NaCl, dried ( $\text{MgSO}_4$ ) and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel (90/10 – ethyl acetate/hexane) affording, GS-277771, oxime 4 (0.104 g, 85%) as a solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.13 (s, 1H), 7.72 (d, 2H), 7.51 (d, 2H), 7.27 (d, 2H), 7.00 (d, 2H), 5.67 (d, 1H), 5.02 (m, 2H), 4.54 (d, 2H), 4.21 (m, 4H), 3.92 (m, 1H), 3.89 (s, 3H), 3.88 (m, 1H), 3.97-3.71 (m, 2H), 3.85-3.70 (m, 2H), 3.16-2.99 (m, 2H), 3.16-2.81 (m, 7H), 1.84 (m, 1H), 1.64-1.48 (m, 2H), 1.37 (t, 6H), 0.94-0.90 (dd, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  20.0; MS (ESI) 756 (M+H).

Scheme 1

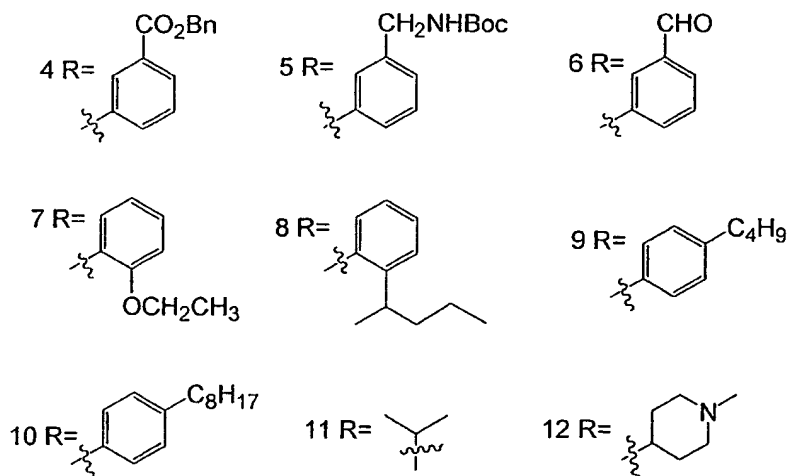




Scheme 1



I. Ethyl(S)-(-)-lactate/Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate/  
DIPEA/EtOAc; II.  $H_2$ /20%Pd-C/EtOAc-EtOH; III. ROH/Benzotriazol-1-  
yloxytripyrrolidinophosphonium hexafluorophosphate/ DIPEA/EtOAc



Example 1

Compound 1 was prepared according to methods from previous Schemes

Example 2

- i Compound 2: To a solution of compound 1 (5.50 g, 7.30 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (5.70g, 10.95 mmol), and Ethyl(S)-(-)lactate (1.30 g, 10.95 mmol) in DMF (50 mL) was added Diisopropylethylamine (5.08 mL, 29.2 mmol). The mixture was stirred for 7 hours after which was diluted in EtOAc. The organic phase was washed with H<sub>2</sub>O (5X), brine, dried over MgSO<sub>4</sub> and *concentrated in vacuo*. The residue was purified by silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Isopropanol= 100/4) to give 3.45 g of compound 2.

Example 3

Compound 3: To the mixture of compound 2 (3.45 g) in EtOH/EtOAc (300 mL/100 mL) was added 20% Pd/C (0.700 g). The mixture was hydrogenated for 1 hour. Celite was added and the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and washed with ethanol. Concentration gave 2.61 g of compound 3.

Example 4

Compound 4: To a solution of compound 3 (1.00 g, 1.29 mmol) in dry dimethylformamide (5 mL) was added 3-Hydroxy-benzoic acid benzyl ester (0.589 g, 2.58 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.34 g, 2.58 mmol), followed by addition of Diisopropylethylamine (900 µL, 5.16 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Isopropanol= 100/3) to provide 67.3 mg of compound 4: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.91 (2H, d, J=8.9 Hz), 7.75 (2H, m), 7.73-7.3 (13H, m), 7.25 (2H, m), 7.21-6.7 (6H, m), 5.87 (1H, m), 5.4-4.8 (6H, m), 4.78-4.21 (4H, m), 3.98 (3H, s), 2.1-1.75 (8H, m), 1.55 (3H, m), 1.28 (3H, m), 0.99 (6H, m).

Example 5

Compound 5: To a solution of compound 3 (1.40 g, 1.81 mmol) in dry dimethylformamide (5 mL) was added (4-Hydroxy-benzyl)-carbamic acid tert-butyl ester (0.80 g, 3.62 mmol),

Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.74 g, 3.62 mmol), followed by addition of Diisopropylethylamine (1.17 ml, 7.24 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ( $\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/3.5$ ) to provide 770 mg of compound 5:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.8(2H, d,  $J=8.9\text{Hz}$ ), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-5.1(2H, m), 4.6-4.23 (4H, m), 3.98 (3H, s), 3.7-2.6 (15H, m), 2.2-1.8 (12H, m), 1.72 (3H, s), 1.58(3H, m), 1.25 (3H, m), 0.95 (6H, m).

#### Example 6

Compound 6: To a solution of compound 3 (1.00 g, 1.29 mmol) in dry dimethylformamide (6 mL) was added 3-Hydroxybenzaldehyde (0.320 g, 2.60 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.35 g, 2.60 mmol), followed by addition of Diisopropylethylamine (901  $\mu\text{L}$ , 5.16 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ( $\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/5$ ) to provide 880 mg of compound 6.

#### Example 7

Compound 7: To a solution of compound 3 (150 mg, 0.190 mmol) in dry dimethylformamide (1 mL) was added 2-Ethoxy-phenol (48.0  $\mu\text{L}$ , 0.380 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (198 mg, 0.380 mmol), followed by addition of Diisopropylethylamine (132  $\mu\text{L}$ , 0.760 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ( $\text{CH}_2\text{Cl}_2/\text{Isopropanol}=100/4$ ) to provide 84.7 mg of compound 7:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (2H, d,  $J=8.9\text{ Hz}$ ), 7.15 (2H, m), 7.01-6.9 (8H, m), 5.66 (1H, m), 5.22-5.04 (2H, m), 4.56- 4.2 (6H, m), 4.08 (2H, m), 3.89 (3H, m), 3.85-3.69 (6H, m), 3.17-2.98 (7H, m), 2.80(3H, m) 1.86 (1H, m), 1.65(2H, m), , 1.62-1.22 (6H, m), 0.92(6H, m).

#### Example 8

Compound 8: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 2-(1-methylbutyl) phenol (21.2 mg, 0.130 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition of Diisopropylethylamine (45.0  $\mu$ L, 0.260 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 8.20 mg of compound 8:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (2H, d,  $J=8.9$  Hz), 7.25 (2H, m), 7.21-6.89 (8H, m), 5.7(1H, m), 5.29-4.9 (2H, m), 4.56- 4.2 (6H, m), 3.89 (3H, m), 3.85-3.69 (6H, m), 3.17-2.89 (8H, m), 2.85(3H, m), 2.3-1.65(4H, m), 1.55-1.35 (6H, m), 0.92(6H, m).

#### Example 9

Compound 9: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 4-N-Butylphenol (19.4 mg, 0.130 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition (45.0  $\mu$ L, 0.260 mmol) of Diisopropylethylamine. The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 9.61 mg of compound 9:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.8(2H, d,  $J=8.9$  Hz), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-4.5 (4H, m), 4.3-3.4.1 (4H, m), 3.9 (3H, m), 3.3-2.59 (11H, m), 2.25 (2H, m), 1.85-1.5 (5H, m), 1.4-1.1(10H, m), 0.95(9H, m).

#### Example 10

Compound 10: To a solution of compound 3 (50.0 mg, 0.0650 mmol) in dry dimethylformamide (1 mL) was added 4-Octylphenol (26.6 mg, 0.130 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (67.1 mg, 0.130 mmol), followed by addition of Diisopropylethylamine (45.0  $\mu$ L, 0.260 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 7.70 mg of compound 10:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.75 (2H, d,  $J=8.9$  Hz), 7.3 (2H, m), 7.2-6.8 (8H, m), 5.70 (1H, m), 5.3-4.9 (4H, m), 4.6- 3.9 (4H, m),

3.89 (3H, m), 3.85-2.59 (12H, m), 2.18-1.75 (10H, m), 1.69-1.50 (8H, m), 1.4-1.27(6H,m), 0.95(9H, m).

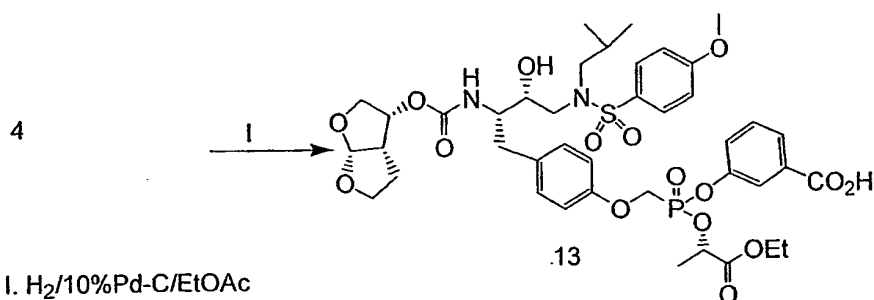
#### Example 11

Compound 11: To a solution of compound 3 (100 mg, 0.120 mmol) in dry dimethylformamide (1 mL) was added Isopropanol (20.0  $\mu$ L, 0.240 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (135 mg, 0.240 mmol), followed by addition of Diisopropylethylamine (83.0  $\mu$ L, 0.480 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography ( $\text{CH}_2\text{Cl}_2$ /Isopropanol= 100/4) to provide 12.2 mg of compound 11:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (2H, d,  $J=8.9$  Hz), 7.15 (2H, m), 7.0 (2H, m), 6.89 (2H, m), 5.65 (1H, m), 5.03-4.86(4H, m), 4.34-4.19 (3H, m), 3.89 (3H, s), 3.88 (1H, m), 3.82 (2H, m), 3.65 (4H, m), 3.2-2.9 (11H, m), 2.80(3H, m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.30(3H,m), 0.92(6H, m).

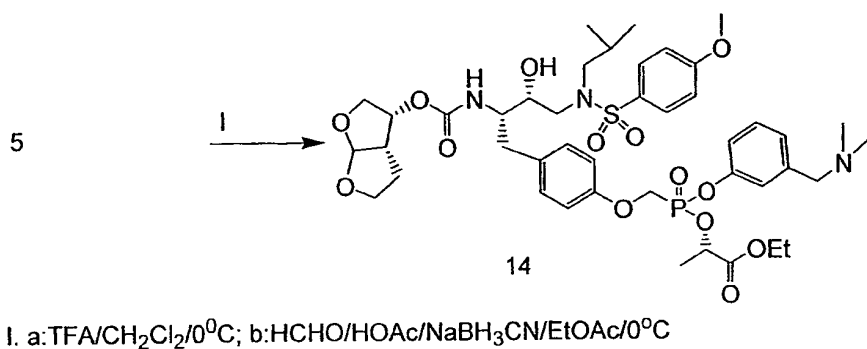
#### Example 12

Compound 12: To a solution of compound 3 (100 mg, 0.120 mmol) in dry dimethylformamide (1mL) was added 4-Hydroxy-1-methylpiperidine (30.0 mg, 0.240 mmol), Benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (135 mg, 0.240 mmol), followed by addition of Diisopropylethylamine (83.0  $\mu$ L, 0.480 mmol). The mixture was stirred for 14 hours, the resulting residue was diluted in EtOAc, washed with brine (3x) and dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by reversed phase HPLC to provide 50.1 mg of compound 12:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.73 (2H, d,  $J=8.9$  Hz), 7.18 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 5.67 (1H, m), 5.2-4.9 (4H, m), 4.30-4.11 (4H, m), 3.98 (1H, m), 3.89 (3H, s), 3.87 (1H, m), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (14H, m), 2.80(3H, m) 1.65(2H, m), 1.86 (1H, m), 1.6(3H, m), 1.30(3H,m), 0.92(6H, m).

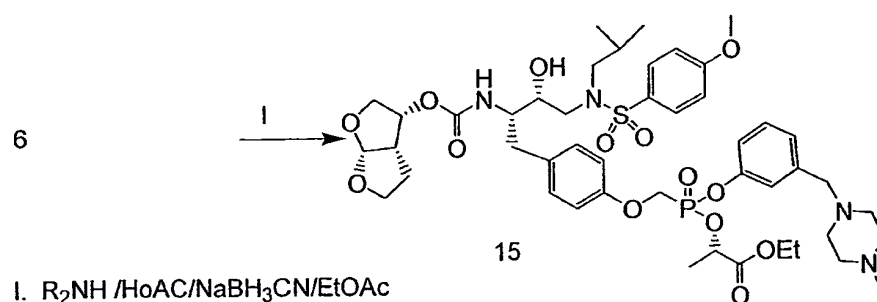
## Scheme 2



## Scheme 3



## Scheme 4



## Example 13

Compound 13: To a solution of compound 4 (4.9 g) in EtOAc (150ml) was added 20% Pd/C (0.90 g), the reaction mixture was hydrogenated for 1 hour. Celite was added and the mixture was stirred for 10 minutes. The mixture was filtered through a pad of celite and washed with ethanol. Concentration gave 4.1 g of compound 13:  $^1\text{H NMR (CDCl}_3)$   $\delta$  7.91 (2H, d,  $J=8.9$  Hz),

7.75 (2H, m), 7.73-7.3 (8H, m), 7.25 (2H, m), 7.21-6.7 (6H, m), 5.4-4.8 (6H, m), 4.78-4.21 (4H, m), 3.98 (3H, s), 2.1-1.75 (8H, m), 1.55 (3H, m), 1.28 (3H, m), 0.99 (6H, m).

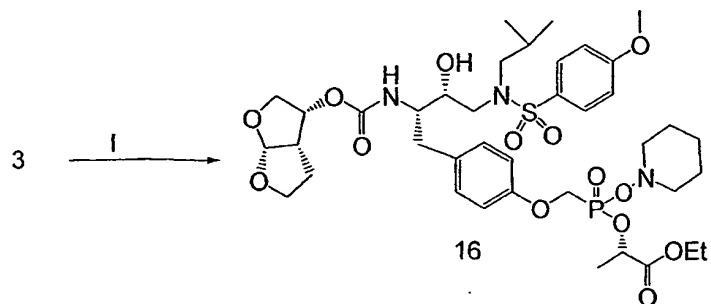
#### Example 14

Compound 14: To a solution of compound 5 (0.770 g, 0.790 mmol) in dichloromethane (10 mL), under ice-cooling, was added trifluoroacetic acid (5 mL), the resulting mixture was stirred at 25°C for two hours. The reaction mixture was concentrated under reduced pressure and the residue was co-evaporated with EtOAc to provide an yellow oil. To a solution of the above oil in (10 mL) of EtOAc, under ice-cooling and stirring was added formaldehyde (210 µL, 2.86 mmol), acetic acid (252 µL, 4.30 mmol), followed by sodium cyanoborohydride (178 mg, 2.86 mmol). The mixture was further stirred at 25°C for 2 hours. The above mixture was concentrated and diluted with EtOAc and washed with H<sub>2</sub>O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using reversed-phase HPLC to provide 420 mg of compound 14: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.8 (2H, d, J=8.9 Hz), 7.4 (2H, m), 7.3-6.8 (8H, m), 5.75 (1H, m), 5.3-5.1 (2H, m), 4.6-4.23 (4H, m), 3.98 (3H, s), 3.7-2.6 (15H, m), 2.2-1.8 (8H, m), 1.72 (3H, s), 1.58 (3H, m), 1.25 (3H, m), 0.95 (6H, m).

#### Example 15

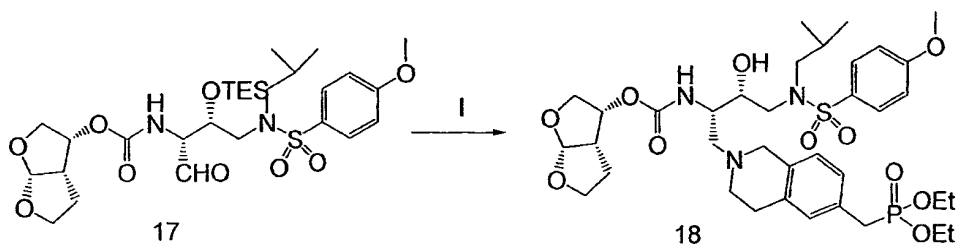
Compound 15: To a solution of compound 6 (100 mg, 0.114 mmol) in EtOAc (1 mL) was added 1-Methyl-piperazine (63.2 mg, 0.570 mmol), acetic acid (34.0 µl, 0.570 mmol) followed by Sodium Cyanoborohydride (14.3 mg, 0.228 mmol). The mixture was stirred at 25°C for 14 hours. The reaction mixture was concentrated and diluted with EtOAc and washed with H<sub>2</sub>O (5X), brine (2x), dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Isopropanol= 100/6.5) to give 5.22 mg of compound 15: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.73 (2H, d, J=8.9 Hz), 7.4-7.18 (8H, m), 7.1-6.89 (2H, m), 5.67 (1H, m), 5.2-4.9 (4H, m), 4.30-4.11 (4H, m), 3.98 (1H, m), 3.89 (3H, s), 3.87 (1H, m), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80-2.25 (8H, m), 1.65 (2H, m), 1.86 (1H, m), 1.6 (3H, m), 1.30 (3H, m), 0.92 (6H, m).

## Scheme 5



I. Piperidin-1-ol/DCC/Pyridine

## Scheme 6

I. a: R<sub>2</sub>NH /HOAc/NaBH<sub>3</sub>CN/EtOAc b: 2%HF/CH<sub>3</sub>CN

## Example 16

Compound 16: To a solution of compound 3 (100mg, 0.120 mmol) in Pyridine (600  $\mu$ L) was added Piperidin-1-ol (48.5 mg, 0.480 mmol), followed by N,N-Dicyclohexylcarbodiimide (99.0 mg, 0.480 mmol). The mixture was stirred for 6 hours, the solvent was concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Methanol= 100/5) to provide 17 mg of compound 16: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.73 (2H, d, J=8.9 Hz), 7.16 (2H, m), 7.0 (2H, m), 6.9 (2H, m), 5.68 (1H, m), 5.17 (1H, m), 5.04 (1H, m), 4.5-4.2 (4H, m), 3.90 (3H, s), 3.75 (2H, m), 3.5-3.3 (4H, m), 3.2-2.9 (10H, m), 2.80 (3H, m), 1.65 (2H, m), 1.86 (1H, m), 1.6 (3H, m), 1.5-1.27 (9H, m), 0.92 (6H, m).

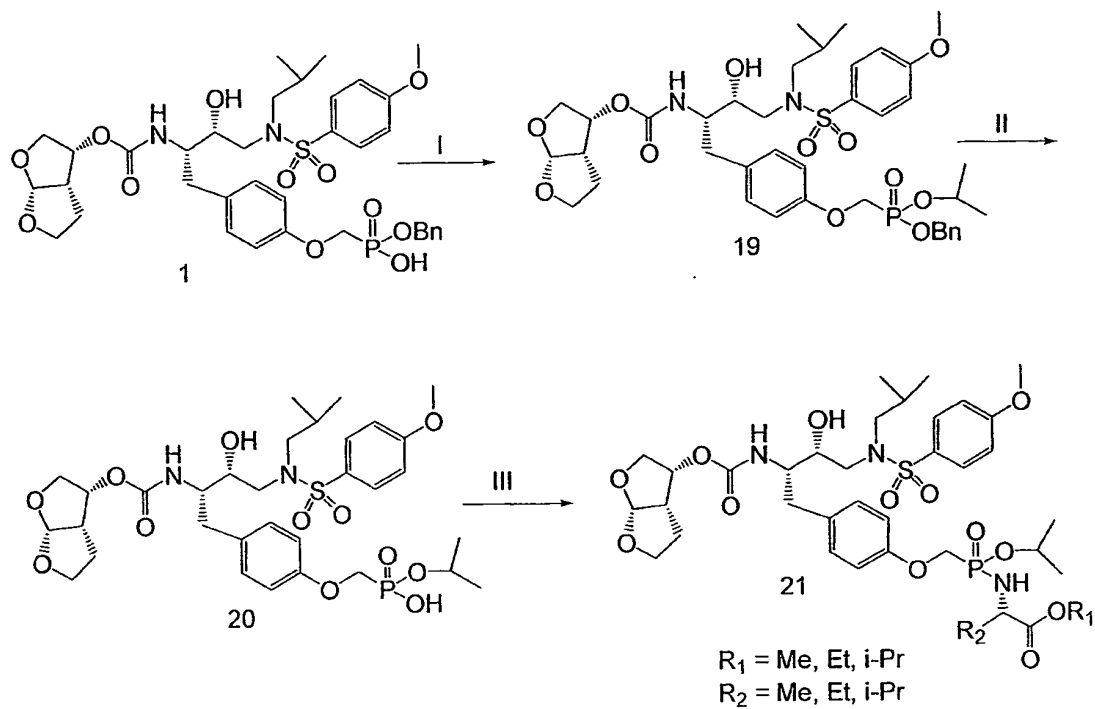


Example 17

Compound 18: To a solution of compound 17 (148 mg, 0.240 mmol) in 4 mL of Methanol was added (1,2,3,4-Tetrahydro-isoquinolin-6-ylmethyl)-phosphonic acid diethyl ester (70.0 mg, 0.240 mmol), acetic acid (43.0  $\mu$ L, 0.720 mmol). The reaction mixture was stirred for 3 minutes, followed by addition of Sodium Cyanoborohydride (75.3 mg, 1.20 mmol). The reaction mixture was stirred at 25°C for 14 hours. The reaction mixture was diluted with EtOAc and washed with H<sub>2</sub>O (3X), brine, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Isopropanol= 100/5) to give 59 mg of TES protected intermediate.

83  $\mu$ L of 48% HF solution was added to acetonitrile (4 mL) to prepare the 2% HF solution. The above 2% HF solution was added to TES protected intermediate (47 mg, 0.053 mmol) and the reaction mixture was stirred for 2 hours. The solvent was concentrated and the residue was diluted with EtOAc, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (CH<sub>2</sub>Cl<sub>2</sub>/Methanol= 100/10) to give 35.2 mg of compound 18: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.73 (2H, d, J=8.9 Hz), 7.05 (2H, m), 6.89 (2H, m), 6.76 (1H, m), 5.75 (1H, m), 5.67 (1H, m), 5.3 (2H, m), 4.2-3.6 (12 H, m), 3.4-2.4 (11 H, m), 2.1-1.8 (6H, m), 1.4-1.28 ( 8 H, m), 0.92(6H, m).

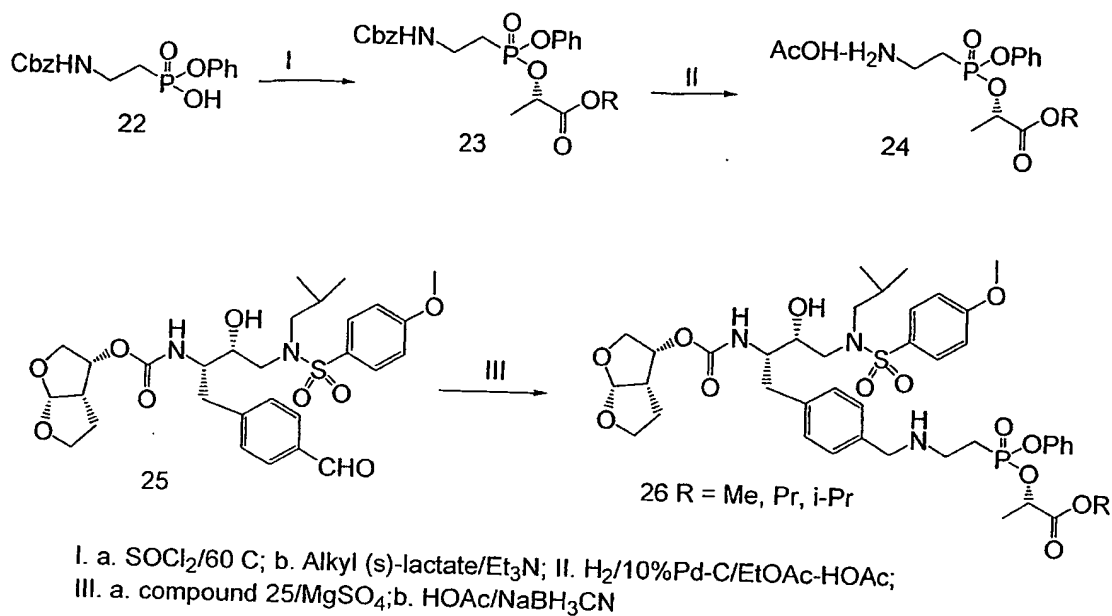
Scheme 7



I. Isopropanol/Benzotriazol-1-yloxytripyrrolidinophosphonium  
 hexafluorophosphate/ DIPEA/DMF;  
 II.  $\text{H}_2$ /10%Pd-C/EtOAc-EtOH;  
 III.  $\text{RNH}_2$ /Aldrithiol-2/ $\text{PPh}_3$ / $\text{iPr}_2\text{NEt}$ /pyridine

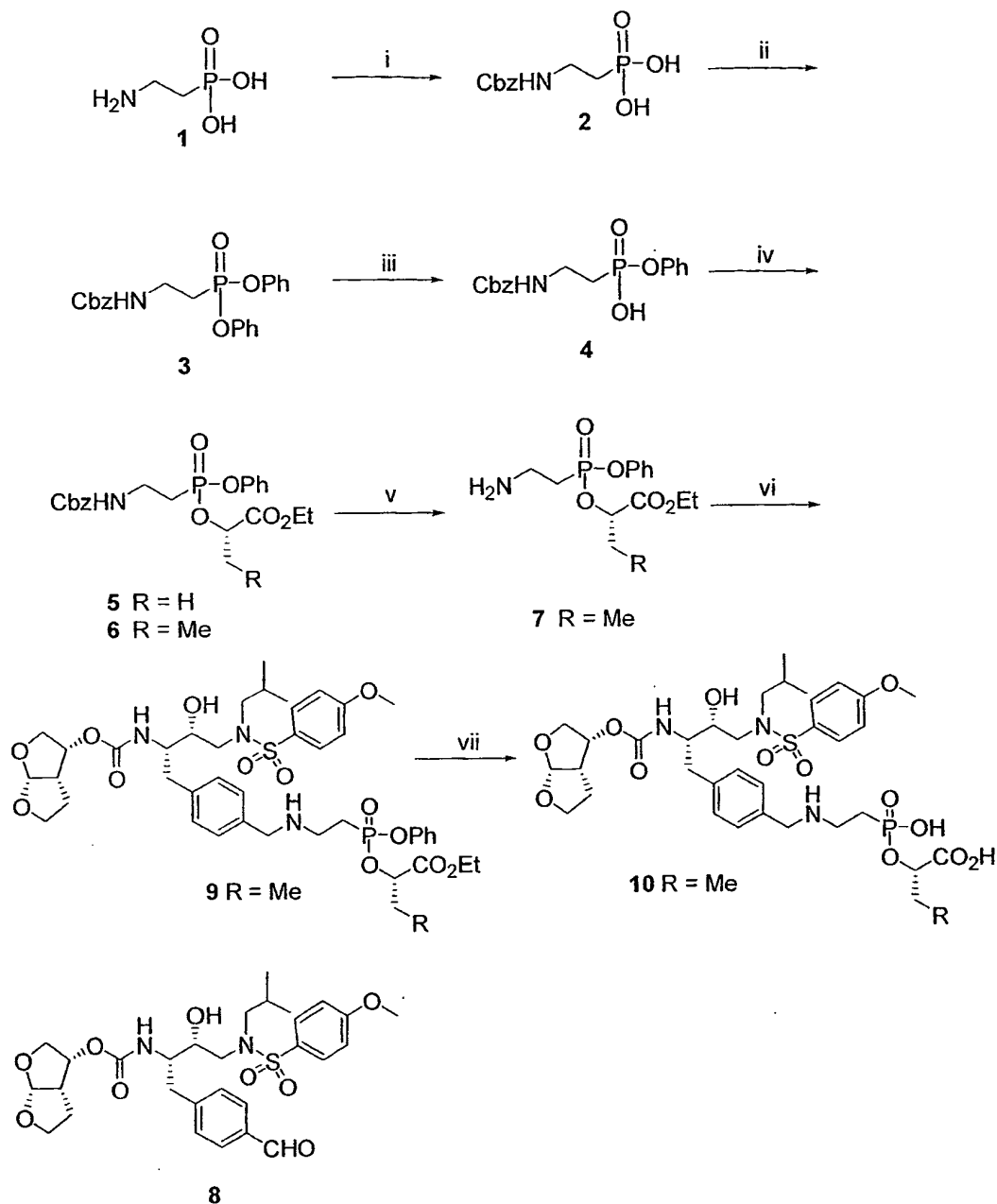
Compound 19 is prepared following the procedure for compound 2 by using monoacid 1.  
 Compound 20 is made following a hydrogenation of compound 19. Mono acid 20 reacts with  
 corresponding amino esters in the presence of Aldrithiol-2 and triphenylphosphine to form  
 compound 21.

Scheme 8



Monoacid 22 is treated with thionyl chloride at  $60^\circ\text{C}$  to form monochloridate, which reacts with corresponding alkyl (s)lactate to generate monolactate 23. Monolactate 23 is hydrogenated with 10%Pd-C in the presence of acetic acid to form amine 24. Aldehyde 25 reacts with amine 24 in the presence of  $\text{MgSO}_4$  to form the intermediate imine, which is reduced with sodium cyanoborohydride to afford compound 26.

Scheme 1



**Reagents and conditions:** i. CbzCl, NaOH, toluene/H<sub>2</sub>O, 100%; ii. a. SOCl<sub>2</sub>, DMF, toluene, 65°C; b. PhOH, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 71%; iii. aq. NaOH, CH<sub>3</sub>CN, 79%; iv. a. SOCl<sub>2</sub>, DMF, toluene, 65°C; b. ethyl lactate, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, (5) 85%; 2-hydroxy butyric acid ethyl ester, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, (6) 75%; v. H<sub>2</sub>, AcOH, 10% Pd/C, EtOH, 94%; vi. a. 7 + 8, 1,2-DCE, MgSO<sub>4</sub>; b. NaBH<sub>3</sub>CN, AcOH, 50%; vii. pig liver esterase, 20% DMSO/PBS, 40°C, 25%.

Example 1

Compound 2: A 3L, 3-neck flask was equipped with a mechanical stirrer and addition funnel and charged with 2-aminoethyl phosphonic acid (60.0g, 480 mmol). 2N Sodium hydroxide (480 mL, 960 mmol) was added and flask cooled to 0°C. Benzyl chloroformate (102.4 g, 600 mmol) in toluene (160mL) was added dropwise with vigorous stirring. The reaction mixture was stirred at 0°C for 30 minutes, then at room temperature for 4 h. 2N sodium hydroxide (240 mL, 480 mmol) was added, followed by benzyl chloroformate (20.5 g, 120 mmol) and the reaction mixture was vigorously stirred for 12 h. The reaction mixture was washed with diethyl ether (3x). The aqueous layer was acidified to pH 2 with concentrated HCl to give a white precipitate. Ethyl acetate was added to the mixture and concentrated HCl (80 mL, 960 mmol) was added. The aqueous layer was extracted with ethyl acetate and combined organic layer was dried (MgSO<sub>4</sub>) and concentrated to give a waxy, white solid (124 g, 479 mmol, 100%). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 7.45-7.30 (m, 5 H, Ar), 5.06 (d, *J* = 14.7 Hz, 2 H, CH<sub>2</sub>Ph), 3.44-3.31 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>), 2.03-1.91 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>P); <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>OD): δ 26.3.

Example 2

Compound 3: To a mixture of compound 2 (50.0 g, 193 mmol) in toluene (1.0 L) was added DMF (1.0 mL) followed by thionyl chloride (56 mL, 768 mmol). The reaction mixture was heated at 65°C for 3-4 h under a stream of argon. The reaction mixture was cooled to room temperature and concentrated. Residual solvent was removed under high vacuum for 1 h. The residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (1.0 L) and cooled to 0°C. Triethylamine (161 mL, 1158 mmol) was added, followed by phenol (54.5 g, 579 mmol). The reaction mixture was warmed to room temperature overnight, then washed with 1.0N HCl, saturated NaHCO<sub>3</sub> solution, brine and dried (MgSO<sub>4</sub>). Concentrated and purified (silica gel, 1:1 EtOAc/Hex) to give a pale yellow solid (56 g, 136 mmol, 71%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.40-7.10 (m, 15 H, Ar), 5.53 (br s, 1 H, NH), 5.11 (br s, 2 H, CH<sub>2</sub>Ph), 3.72-3.60 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>), 2.49-2.30 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>P); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 22.9.

Example 3

Compound 4: To a solution of compound 3 (64 g, 155.6 mmol) in acetonitrile (500 mL) at 0°C was added 2.0M sodium hydroxide. The reaction mixture was stirred at 0°C for 30 min, then at room temperature for 2.5 h. The reaction mixture was concentrated to 100 mL and diluted with H<sub>2</sub>O (500 mL). The aqueous solution was washed with EtOAc (3 x 300 mL). The aqueous layer was acidified to pH 1 with concentrated HCl, producing a white precipitate. The mixture was extracted with EtOAc (4 x 300 mL) and combined organic layer was washed with brine and dried (MgSO<sub>4</sub>). Concentration gave a solid, which was recrystallized from hot EtOAc (450 mL) to give a white solid (41.04 g, 122 mmol, 79%). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 7.45-7.10 (m, 10 H, Ar), 5.09 (s, 2 H, CH<sub>2</sub>Ph), 3.53-3.30 (m, 2 H, NCH<sub>2</sub>CH<sub>2</sub>), 2.25-2.10 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>P); <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>OD): δ 24.5.

#### Example 4

Compound 5: To a mixture of compound 4 (28 g, 83 mmol) in toluene (500 mL) was added DMF (1.0 mL), followed by thionyl chloride (36.4 mL, 499 mmol). The mixture was heated at 65°C for 2 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous CH<sub>2</sub>Cl<sub>2</sub> (350 mL) and cooled to 0°C. Triethylamine (45.3 mL, 332 mmol) was added slowly, followed by the dropwise addition of ethyl lactate (18.8 mL, 166 mmol). The reaction mixture was stirred at 0°C for 30 min, then warmed to room temperature overnight. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with 1 N HCl, saturated NaHCO<sub>3</sub> solution, brine and dried (MgSO<sub>4</sub>). Concentration and purification (silica gel, 1:5 to 1:0 EtOAc/Hex) gave a pale yellow oil (30.7 g, 71 mmol, 85%) as a mixture of diastereomers which were separated by HPLC (Dynamax reverse phase C-18 column, 60% acetonitrile/H<sub>2</sub>O). More polar diastereomer: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.40-7.10 (m, 10 H, Ar), 5.65 (s, 1 H, NH), 5.12 (s, 2 H, CH<sub>2</sub>Ph), 5.10-5.00 (m, 1 H, OCHC) 4.17 (q, *J* = 6.9 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 3.62 (dt, *J*<sub>1</sub> = 20.4 Hz, *J*<sub>2</sub> = 6.0 Hz, 2 H, NCH<sub>2</sub>CH<sub>2</sub>), 2.25 (dt, *J*<sub>1</sub> = 18.0 Hz, *J*<sub>2</sub> = 6.0 Hz, 2 H, CH<sub>2</sub>CH<sub>2</sub>P), 1.60 (dd, *J*<sub>1</sub> = *J*<sub>2</sub> = 6.9 Hz, 3 H, CHCH<sub>3</sub>), 1.23 (t, *J* = 6.9 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 26.2. Less polar diastereomer: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.40-7.10 (m, 10 H, Ar), 5.87 (s, 1 H, NH), 5.13 (s, 2 H, CH<sub>2</sub>Ph), 5.10-5.00 (dq, *J*<sub>1</sub> = *J*<sub>2</sub> = 6.9 Hz, 1 H, OCHC) 4.22 (q, *J* = 7.2 Hz, 2 H, OCH<sub>2</sub>CH<sub>3</sub>), 3.68 (dt, *J*<sub>1</sub> = 21.6 Hz, *J*<sub>2</sub> = 6.9 Hz, 2 H, NCH<sub>2</sub>CH<sub>2</sub>), 2.40-2.20 (m, 2 H, CH<sub>2</sub>CH<sub>2</sub>P), 1.49 (dd, *J*<sub>1</sub> = 70.2 Hz, *J*<sub>2</sub> = 6.9 Hz, 3 H, CHCH<sub>3</sub>), 1.28 (t, *J* = 6.9 Hz, 3 H, OCH<sub>2</sub>CH<sub>3</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 28.3.

Example 5

Compound 6: 2-Hydroxy-butyric acid ethyl ester was prepared as follows: To a solution of L-2-aminobutyric acid (100g, 970 mmol) in 1.0 N  $\text{H}_2\text{SO}_4$  (2 L) at  $0^\circ\text{C}$  was added  $\text{NaNO}_2$  (111 g, 1610 mmol) in  $\text{H}_2\text{O}$  (400 mL) over 2 h. The reaction mixture was stirred at room temperature for 18h. Reaction mixture was extracted with EtOAc (4x) and combined organic layer was dried ( $\text{MgSO}_4$ ) and concentrated to give a yellow solid (41.5 g). This solid was dissolved in absolute ethanol (500 mL) and concentrated HCl (3.27 mL, 39.9 mmol) was added. Reaction mixture was heated to  $80^\circ\text{C}$ . After 24 h, concentrated HCl (3 mL) was added and reaction continued for 24 h. Reaction mixture was concentrated and product was distilled to give a colorless oil (31 g, 235 mmol, 59%).

To a mixture of compound 4 (0.22 g, 0.63 mmol) in anhydrous acetonitrile (3.0 mL) was added thionyl chloride (0.184 mL, 2.52 mmol). The mixture was heated at  $65^\circ\text{C}$  for 1.5 h providing a pale yellow solution. The reaction mixture was concentrated and dried for 45 min under high vacuum. The residue was dissolved in anhydrous  $\text{CH}_2\text{Cl}_2$  (3.3 mL) and cooled to  $0^\circ\text{C}$ . Triethylamine (0.26 mL, 1.89 mmol) was added slowly, followed by the dropwise addition of 2-hydroxy-butyric acid ethyl ester (0.167 mL, 1.26 mmol). The reaction mixture was stirred at  $0^\circ\text{C}$  for 5 min, then warmed to room temperature overnight. The reaction mixture was concentrated, dissolved in EtOAc and washed with 1.0 N HCl, saturated  $\text{NaHCO}_3$  solution, brine and dried ( $\text{MgSO}_4$ ). Concentration and purification (silica gel, 3:2 EtOAc/Hex) gave a pale yellow oil (0.21 g, 0.47 mmol, 75%). For major diastereomer,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.35-7.10 (m, 10 H, Ar), 5.91 (s, 1 H, NH), 5.12 (s, 2 H,  $\text{CH}_2\text{Ph}$ ), 4.94-4.83 (m, 1 H, OCHC), 4.27-4.12 (m, 2 H,  $\text{OCH}_2\text{CH}_3$ ), 3.80-3.50 (m, 2 H,  $\text{NCH}_2\text{CH}_2$ ), 2.39-2.19 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.82-1.71 (m, 2 H,  $\text{CHCH}_2\text{CH}_3$ ), 1.30-1.195 (m, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 0.81 (t,  $J = 7.5$  Hz, 3 H,  $\text{CHCH}_2\text{CH}_3$ );  $^{31}\text{P}$  NMR (120 MHz,  $\text{CDCl}_3$ ):  $\delta$  28.3. For minor diastereomer,  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.35-7.10 (m, 10 H, Ar), 5.74 (s, 1 H, NH), 5.11 (s, 2 H,  $\text{CH}_2\text{Ph}$ ), 4.98-4.94 (m, 1 H, OCHC), 4.27-4.12 (m, 2 H,  $\text{OCH}_2\text{CH}_3$ ), 3.80-3.50 (m, 2 H,  $\text{NCH}_2\text{CH}_2$ ), 2.39-2.19 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.98-1.82 (m, 2 H,  $\text{CHCH}_2\text{CH}_3$ ), 1.30-1.195 (m, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 1.00 (t,  $J = 7.5$  Hz, 3 H,  $\text{CHCH}_2\text{CH}_3$ );  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  26.2.

Example 6

Compound 7: A mixture of compound 6, (0.53 g, 1.18 mmol) acetic acid (0.135 mL, 2.36 mmol) and 10% palladium on activated carbon (0.08 g) in absolute ethanol (12 mL) was stirred under a hydrogen atmosphere (1 atm) for 3 h. Reaction mixture was filtered through Celite, concentrated, and resubjected to identical reaction conditions. After 2 h, Celite was added to the reaction mixture and mixture was stirred for 2 min, then filtered through a pad of Celite and concentrated. Dried under high vacuum to give the diastomeric acetate salt as a oil (0.42 g, 1.11 mmol, 94%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.40-7.10 (m, 5 H, Ar), 5.00-4.80 (m, 1 H,  $\text{OCHC}$ ), 4.28-4.10 (m, 2 H,  $\text{OCH}_2\text{CH}_2$ ), 3.32-3.14 (m, 2 H,  $\text{NCH}_2\text{CH}_2$ ), 2.45-2.22 (m, 2 H,  $\text{CH}_2\text{CH}_2\text{P}$ ), 1.97 (s, 3 H, Ac), 1.97-1.70 (m, 2 H,  $\text{CHCH}_2\text{CH}_3$ ), 1.30-1.18 (m, 3 H,  $\text{OCH}_2\text{CH}_3$ ), 1.00 (t,  $J = 7.5$  Hz, 1 H,  $\text{CHCH}_2\text{CH}_3$ ), 0.80 (t,  $J = 7.5$  Hz, 2 H,  $\text{CHCH}_2\text{CH}_3$ );  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  27.6 (major, 1.85), 26.0 (minor, 1.01).

#### Example 7

Compound 9: A solution of aldehyde 8 (0.596 g, 1.01 mmol) and compound 7 (0.42 g, 1.11 mmol) were stirred together in 1,2-dichloroethane (4.0 mL) in the presence of  $\text{MgSO}_4$  for 3 h. Acetic acid (0.231 mL, 4.04 mmol) and sodium cyanoborohydride (0.127 g, 2.02 mmol) were added and reaction mixture was stirred for 50 min at room temperature. Reaction mixture was quenched with saturated  $\text{NaHCO}_3$  solution, diluted with EtOAc, and vigorously stirred for 5 min. Brine was added and extracted with EtOAc (2x). Combined organic layer was dried ( $\text{MgSO}_4$ ) concentrated and purified (silica gel, EtOAc, then 10% EtOH/EtOAc) to give a colorless foam. Acetonitrile (4 mL) and trifluoroacetic acid (0.06 mL) were added and concentrated to a volume of 1 mL.  $\text{H}_2\text{O}$  (10 mL) was added and lyophilized to give the TFA salt as a white powder (0.51 g, 0.508 mmol, 50%).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  7.79 (d,  $J = 8.4$  Hz, 2 H,  $(\text{SO}_2\text{C}(\text{CH}_2)_2$ ), 7.43-7.20 (m, 9 H, Ar), 7.10 (d,  $J = 8.4$  Hz, 2 H,  $(\text{CH}_2\text{COCH}_3)$ ), 5.85 (d,  $J = 8.4$  Hz, 1 H,  $\text{NH}$ ), 5.55 (d,  $J = 4.5$  Hz, 1 H,  $\text{OCHO}$ ), 5.00-4.75 (m, 2 H,  $\text{CH}_2\text{CHOC}(\text{O})$ ,  $\text{POCHC}$ ), 4.39-4.05 (m, 2 H,  $\text{PhCH}_2\text{N}$ ,  $\text{OCH}_2\text{CH}_3$ ), 3.89 (s, 3 H,  $\text{OCH}_3$ ), 3.88-3.30 (m, 9H), 3.15-2.84 (m, 5 H), 2.65-2.42 (m, 3 H), 2.10-1.68 (m, 5 H), 1.65-1.15 (m, 5 H), 1.05-0.79 (m, 9 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  24.8 (major, 1.85), 23.1 (minor, 1.01).

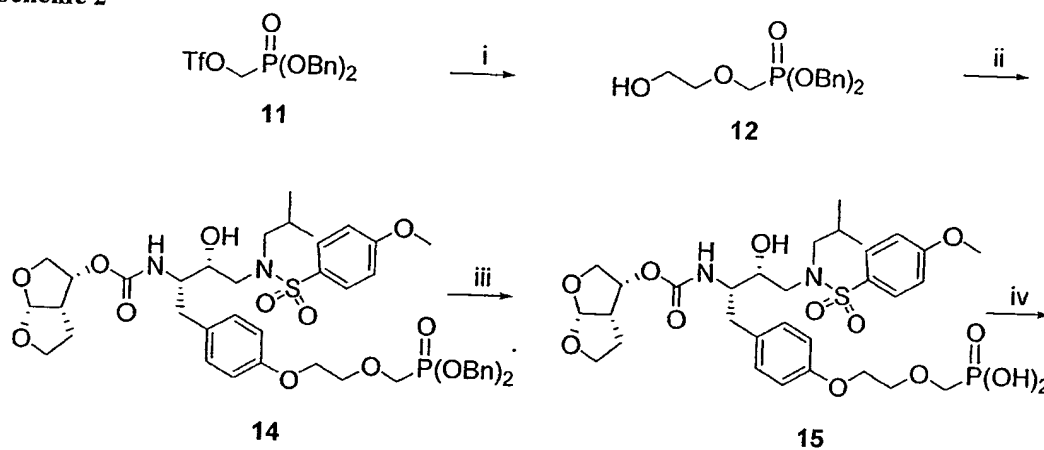
#### Example 8

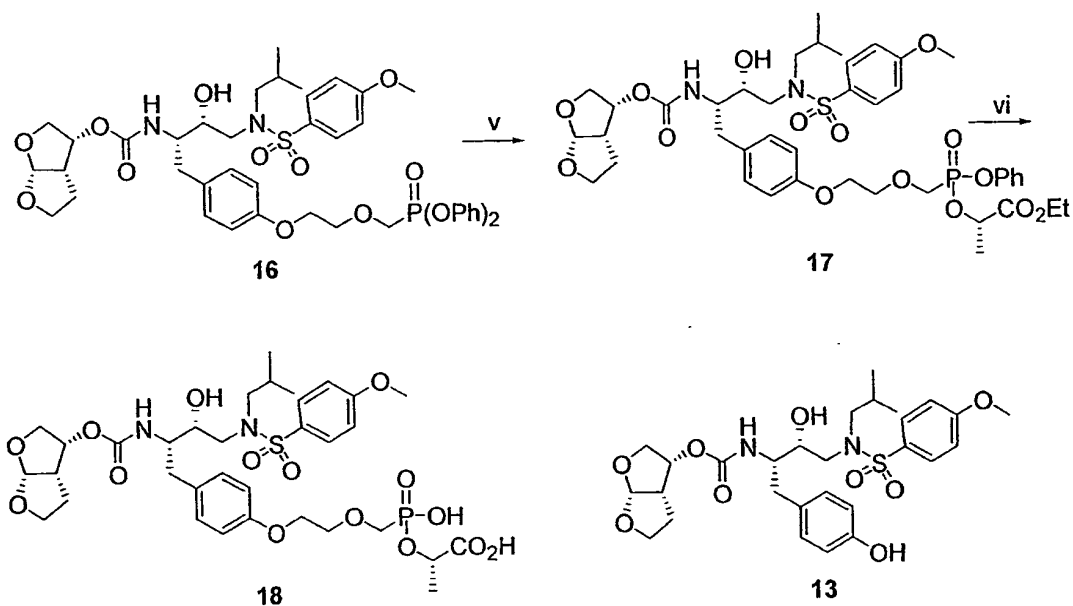
Compound 10: Compound 9 (0.041 g, 0.041 mmol) was dissolved in DMSO (1.9 mL) and to this solution was added phosphate buffered saline, pH 7.4 (10 mL) and pig liver esterase



(Sigma, 0.2 mL). Reaction mixture was stirred for 24 h at 40°C. After 24 h, additional esterase (0.2 mL) was added and reaction was continued for 24 h. Reaction mixture was concentrated, resuspended in methanol and filtered. Filtrate was concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (8 mg, 0.010 mmol, 25%). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD): δ 7.78 (d, *J* = 8.9 Hz, 2 H, (SO<sub>2</sub>C(CH)<sub>2</sub>), 7.43-7.35 (m, 4 H, Ar), 7.11 (d, *J* = 8.9 Hz, 2 H, (CH)<sub>2</sub>COCH<sub>3</sub>), 5.62 (d, *J* = 5.2 Hz, 1 H, OCHO), 4.96-4.77 (m, 2 H, CH<sub>2</sub>CHOC(O), POCHC), 4.21 (br s, 2 H, PhCH<sub>2</sub>N), 3.97-3.70 (m, 6 H), 3.90 (s, 3 H, OCH<sub>3</sub>), 3.50-3.30 (m, 3 H), 3.26-3.02 (m, 2 H), 2.94-2.58 (m, 4 H), 2.09-1.78 (m, 5 H), 1.63-1.52 (m, 2 H), 1.05-0.97 (m, 3 H); 0.94 (d, *J* = 6.7 Hz, 3 H), 0.88 (d, *J* = 6.7 Hz, 3 H); <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>OD): δ 20.8.

Scheme 2





Reagents and conditions: i. ethylene glycol,  $\text{Mg}(\text{OtBu})_2$ , DMF, 48%; ii. a.  $\text{Ti}_2\text{O}$ , 2,6-lutidine,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ ; b. **13**,  $\text{CsCO}_3$ ,  $\text{CH}_3\text{CN}$ ,  $0^\circ\text{C}$  to room temperature, 65%; iii.  $\text{H}_2$ , Pd/C, EtOH, 107%; iv. DCC, PhOH, pyr,  $70^\circ\text{C}$ , 31%; v. a. NaOH,  $\text{CH}_3\text{CN}$ ,  $0^\circ\text{C}$ ; b. DCC, ethyl lactate, pyr,  $70^\circ\text{C}$ , 52%; vi.  $\text{CH}_3\text{CN}$ , DMSO, PBS, porcine liver esterase,  $38^\circ\text{C}$ , 69%.

#### Example 9

Compound 12: To a solution of compound **11** (4.10 g, 9.66 mmol) and anhydrous ethylene glycol (5.39 mL, 96.6 mmol) in anhydrous DMF (30 mL) at  $0^\circ\text{C}$  was added powdered magnesium *tert*-butoxide (2.05 g, 12.02 mmol). The reaction mixture was stirred at  $0^\circ\text{C}$  for 1.5 h, then concentrated. The residue was partitioned between EtOAc and  $\text{H}_2\text{O}$  and washed with 1 N HCl, saturated  $\text{NaHCO}_3$  solution, and brine. Organic layer dried ( $\text{MgSO}_4$ ), concentrated and purified (silica gel, 4% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give a colorless oil (1.55 g, 48%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.37 (s, 10 H, Ar), 5.40-5.05 (m, 4 H,  $\text{CH}_2\text{Ph}$ ), 3.84 (d,  $J = 8.1$  Hz, 2 H,  $\text{PCH}_2\text{O}$ ), 3.70-3.60 (m, 4 H,  $\text{OCH}_2\text{CH}_2\text{O}$ ,  $\text{OCH}_2\text{CH}_2\text{O}$ );  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  22.7.

#### Example 10

Compound 14: To a solution of compound **12** (0.75 g, 2.23 mmol) and 2,6-lutidine (0.78 mL, 6.69 mmol) in  $\text{CH}_2\text{Cl}_2$  (20 mL) at  $-78^\circ\text{C}$  was added trifluoromethanesulfonic anhydride (0.45

mL, 2.68 mmol). The reaction mixture was stirred at  $-78^{\circ}\text{C}$  for 40 min, then diluted with  $\text{CH}_2\text{Cl}_2$  and washed with 1 N HCl, saturated  $\text{NaHCO}_3$  and dried ( $\text{MgSO}_4$ ). Concentration gave a yellow oil that was dissolved in anhydrous acetonitrile (20 mL). Phenol 13 (1.00 g, 1.73 mmol) was added to the solution, which was cooled to  $0^{\circ}\text{C}$ . Cesium carbonate (0.619 g, 1.90 mmol) was added and reaction mixture was stirred at  $0^{\circ}\text{C}$  for 2 h, then at room temperature for 1.5 h. Additional cesium carbonate (0.200 g, 0.61 mmol) was added and reaction was continued for 1.5 h, then filtered. Concentration of the filtrate and purification (silica gel, 3%  $\text{MeOH}/\text{CH}_2\text{Cl}_2$ ) gave a yellow gum (1.005 g, 65%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.71 (d,  $J = 8.7$  Hz, 2 H,  $\text{SO}_2\text{C}(\text{CH})_2$ ), 7.34 (s, 10 H,  $\text{PhCH}_2\text{O}$ ), 7.11 (d,  $J = 8.1$  Hz, 2 H,  $\text{CH}_2\text{C}(\text{CH})_2(\text{CH})_2$ ), 6.98 (d,  $J = 8.7$  Hz, 2 H,  $(\text{CH})_2\text{COCH}_3$ ), 6.78 (d,  $J = 8.7$  Hz, 2 H,  $(\text{CH})_2\text{COCH}_2$ ), 5.62 (d,  $J = 5.4$  Hz, 1 H,  $\text{OCHO}$ ), 5.16-4.97 (m, 6 H), 4.05-3.65 (m, 12 H), 3.86 (s, 3 H,  $\text{OCH}_3$ ), 3.19-2.66 (m, 7 H), 1.95-1.46 (m, 3 H), 0.92 (d,  $J = 6.6$  Hz, 3 H,  $\text{CH}(\text{CH}_3)_2$ ), 0.88 (d,  $J = 6.6$  Hz, 3 H,  $\text{CH}(\text{CH}_3)_2$ );  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  21.9.

#### Example 11

Compound 15: A mixture of compound 14 (0.410 g, 0.457 mmol) and 10% palladium on carbon (0.066 g) in ethanol (5.0 mL) was stirred under a hydrogen atmosphere (1 atm) for 16 h. Celite was added and the mixture was stirred for 5 min, then filtered through Celite and concentrated to give a foam (0.350 g, 107%).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.76 (d,  $J = 8.7$  Hz, 2 H,  $\text{SO}_2\text{C}(\text{CH})_2$ ), 7.15 (d,  $J = 8.4$  Hz, 2 H,  $\text{CH}_2\text{C}(\text{CH})_2(\text{CH})_2$ ), 7.08 (d,  $J = 8.4$  Hz, 2 H,  $(\text{CH})_2\text{COCH}_3$ ), 6.82 (d,  $J = 8.4$  Hz, 2 H,  $(\text{CH})_2\text{COCH}_2$ ), 5.59 (d,  $J = 5.4$  Hz, 1 H,  $\text{OCHO}$ ), 5.16-4.97 (masked by  $\text{CD}_3\text{OH}$ , 1 H), 4.09-4.02 (m, 2 H), 3.99-3.82 (m, 10 H), 3.88 (s, 3 H,  $\text{OCH}_3$ ), 3.52-3.32 (m, 1 H), 3.21-2.75 (m, 5 H), 2.55-2.40 (m, 1 H), 2.10-1.95 (m, 1 H), 1.75-1.25 (m, 2 H), 0.93 (d,  $J = 6.3$  Hz, 3 H,  $\text{CH}(\text{CH}_3)_2$ ), 0.88 (d,  $J = 6.6$  Hz, 3 H,  $\text{CH}(\text{CH}_3)_2$ );  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  19.5.

#### Example 12

Compound 16: Compound 15 (0.350 g, 0.488 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL), each time filling with  $\text{N}_2$ . Residue was dissolved in anhydrous pyridine (2.5 mL) and phenol (0.459 g, 4.88 mmol) was added. This solution was heated to  $70^{\circ}\text{C}$ , then 1,3-dicyclohexylcarbodiimide (0.403 g, 1.93 mmol) was added and reaction mixture was heated at  $70^{\circ}\text{C}$  for 7 h. Reaction mixture was concentrated, coevaporated with toluene and

residue obtained was diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>, then another column 75% EtOAc/Hex) to give a clear oil (0.1324 g, 31%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.71 (d, *J* = 8.7 Hz, 2 H, SO<sub>2</sub>C(CH)<sub>2</sub>), 7.41-7.18 (m, 10 H, Ar), 7.14 (d, *J* = 8.4 Hz, 2 H, CH<sub>2</sub>C(CH)<sub>2</sub>(CH)<sub>2</sub>), 6.99 (d, *J* = 9.0 Hz, 2 H, (CH)<sub>2</sub>COCH<sub>3</sub>), 6.83 (d, *J* = 8.4 Hz, 2 H, (CH)<sub>2</sub>COCH<sub>2</sub>), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 2 H), 4.32-3.62 (m, 12 H), 3.87 (s, 3 H, OCH<sub>3</sub>), 3.22-2.73 (m, 7 H), 1.95-1.75 (m, 3 H), 0.93 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 14.3.

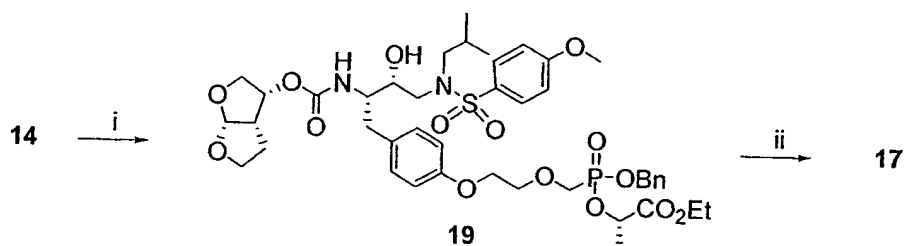
### Example 13

Compound 17: To a solution of compound 16 (0.132 g, 0.152 mmol) in acetonitrile (1.5 mL) at 0°C was added 1.0 M NaOH (0.38 mL, 0.381 mmol). Reaction mixture was stirred for 2 h at 0°C, then Dowex 50 (H<sup>+</sup>) resin was added until pH = 1. The resin was removed by filtration and the filtrate was concentrated and washed with EtOAc/Hex (1:2, 25 mL), then dried under high vacuum to give a clear film (0.103 g, 85%). This film was coevaporated with anhydrous pyridine (3 x 5 mL), filling with N<sub>2</sub>. The residue was dissolved in anhydrous pyridine (1 mL) and ethyl lactate (0.15 mL, 1.30 mmol) was added and reaction mixture was heated at 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (0.107 g, 0.520 mmol) was added and reaction mixture was stirred at 70°C for 2.5 h. Additional 1,3-dicyclohexylcarbodiimide (0.055 g, 0.270 mmol) was added and reaction continued for another 1.5 h. Reaction mixture was concentrated and coevaporated with toluene and diluted with EtOAc, precipitating 1,3-dicyclohexylurea. The mixture was filtered and filtrate concentrated and residue obtained was purified (silica gel, 80 to 100% EtOAc/Hex) to give a white foam (0.0607 g, 52%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.71 (d, *J* = 8.7 Hz, 2 H, SO<sub>2</sub>C(CH)<sub>2</sub>), 7.39-7.16 (m, 5 H, Ar), 7.13 (d, *J* = 8.1 Hz, 2 H, CH<sub>2</sub>C(CH)<sub>2</sub>(CH)<sub>2</sub>), 6.99 (d, *J* = 9.0 Hz, 2 H, (CH)<sub>2</sub>COCH<sub>3</sub>), 6.82 (d, *J* = 8.4 Hz, 2 H, (CH)<sub>2</sub>COCH<sub>2</sub>), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.92 (m, 3 H), 4.35-3.65 (m, 14 H), 3.87 (s, 3 H, OCH<sub>3</sub>), 3.22-2.73 (m, 7 H), 1.95-1.80 (m, 3 H), 1.59 (d, *J* = 6.9 Hz, 1.5 H, CCHCH<sub>3</sub>), 1.47 (d, *J* = 7.2 Hz, 1.5 H, CCHCH<sub>3</sub>), 1.37-1.18 (m, 3 H), 0.92 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 19.2, 17.2.

**Example 14**

Compound 18: Compound 17 (11.5 mg, 0.013 mmol) was dissolved in DMSO (0.14 mL) and acetonitrile (0.29 mL). PBS (pH 7.4, 1.43 mL) was added slowly with stirring. Porcine liver esterase (Sigma, 0.1 mL) was added and reaction mixture was gently stirred at 38°C. After 24 h, additional porcine liver esterase (0.1 mL) and DMSO (0.14 mL) were added and reaction mixture stirred for 48 h at 38°C. Reaction mixture concentrated and methanol was added to precipitate the enzyme. The mixture was filtered, concentrated and purified by reverse phase chromatography to give a white powder after lyophilization (7.1 mg, 69%). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 7.76 (d, *J* = 8.7 Hz, 2 H, SO<sub>2</sub>C(CH<sub>2</sub>)<sub>2</sub>), 7.15 (d, *J* = 8.4 Hz, 2 H, CH<sub>2</sub>C(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 7.08 (d, *J* = 9.0 Hz, 2 H, (CH<sub>2</sub>)<sub>2</sub>COCH<sub>3</sub>), 6.83 (d, *J* = 8.7 Hz, 2 H, (CH<sub>2</sub>)<sub>2</sub>COCH<sub>2</sub>), 5.59 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.16-4.90 (masked by CD<sub>3</sub>OH, 2 H), 4.19-3.65 (m, 12 H), 3.88 (s, 3 H, OCH<sub>3</sub>), 3.50-3.27 (m, 1 H), 3.20-2.78 (m, 5 H), 2.55-2.40 (m, 1 H), 2.05-1.90 (m, 1 H), 1.75-1.30 (m, 2 H), 1.53 (d, *J* = 6.6 Hz, 3 H, CCHCH<sub>3</sub>), 0.93 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.88 (d, *J* = 6.6 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>); <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>OD): δ 16.7.

Alternatively, compound 17 was prepared as described below (Scheme 3).

**Scheme 3**

Reagents and conditions: i. a. 14, DABCO, toluene, reflux; b. ethyl lactate, PyBOP, DIPEA, DMF, 59%; ii. a. H<sub>2</sub>, Pd/C, EtOH; b. PhOH, PyBOP, DIPEA, DMF, 35%.

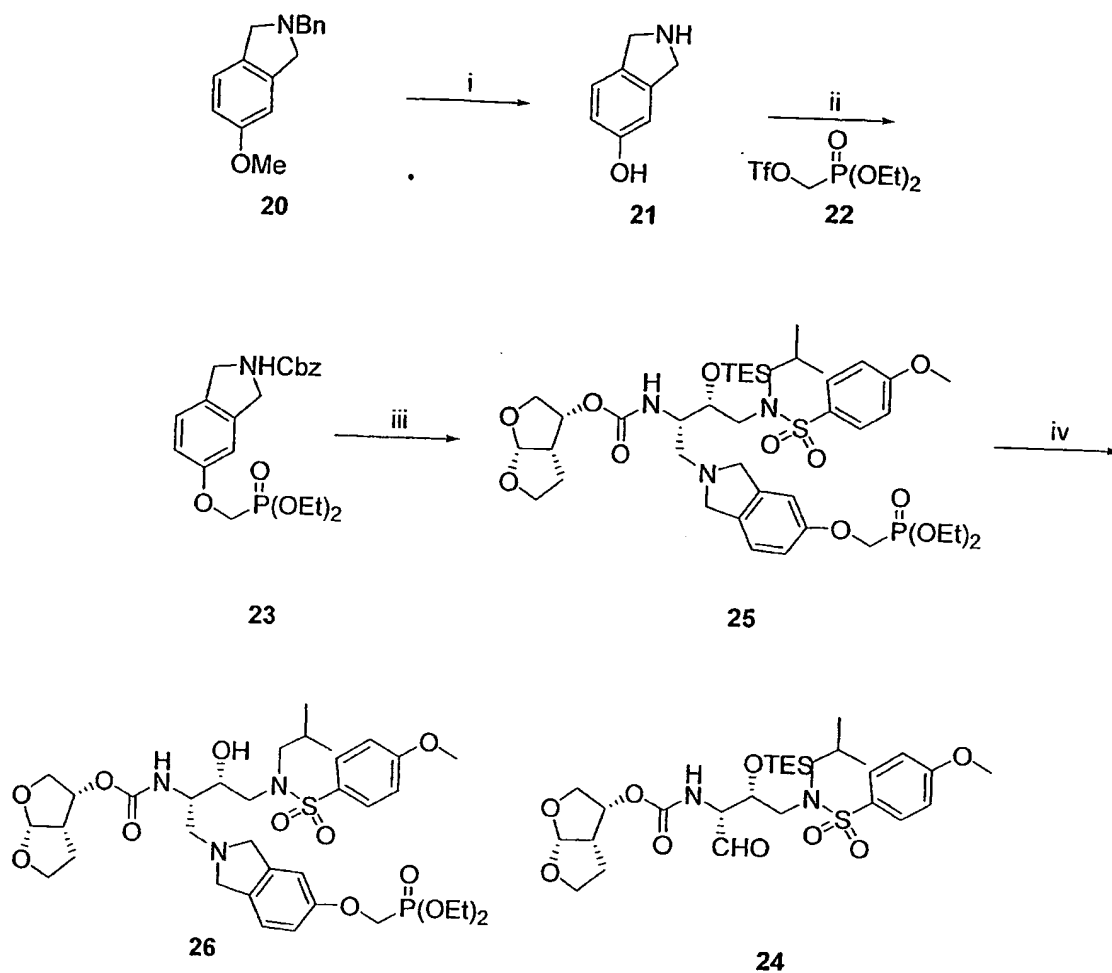
Example 15

Compound 19: To a solution of compound **14** (0.945 g, 1.05 mmol) in anhydrous toluene (10.0 mL) was added 1,4-diazobicyclo[2.2.2] octane (0.130 g, 1.16 mmol) and reaction mixture was refluxed for 2 h. After cooling to room temperature, reaction mixture was diluted with EtOAc and washed with 1.0 N HCl and dried (MgSO<sub>4</sub>). Concentration gave a white foam (0.785 g, 93%). Residue was dissolved in anhydrous DMF (10.0 mL) and to this solution was added ethyl (S)-lactate (0.23 mL, 2.00 mmol) and diisopropylethylamine (0.70 mL, 4.00 mmol), followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (1.041 g, 2.00 mmol). Reaction mixture was stirred for 20 h, then concentrated and residue was dissolved in EtOAc and washed with 1.0 N HCl, saturated NaHCO<sub>3</sub>, brine and dried (MgSO<sub>4</sub>). Concentration and purification (silica gel, 2 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>) gave an off-white foam (0.520 g, 59%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.72 (d, *J* = 7.5 Hz, 2 H, SO<sub>2</sub>C(CH<sub>2</sub>)<sub>2</sub>), 7.50-7.27 (m, 4 H, Ar), 7.12 (d, *J* = 8.1 Hz, 2 H, CH<sub>2</sub>C(CH<sub>2</sub>)<sub>2</sub>(CH<sub>2</sub>)<sub>2</sub>), 7.00 (d, *J* = 6.6 Hz, 2 H, (CH<sub>2</sub>)<sub>2</sub>COCH<sub>3</sub>), 6.81 (d, *J* = 8.4 Hz, 2 H, (CH<sub>2</sub>)<sub>2</sub>COCH<sub>2</sub>), 5.64 (d, *J* = 5.1 Hz, 1 H, OCHO), 5.37-4.90 (m, 5 H), 4.35-3.65 (m, 14 H), 3.88 (s, 3 H, OCH<sub>3</sub>), 3.24-2.70 (m, 7 H), 1.90-1.70 (m, 3 H), 1.54 (d, *J* = 6.9 Hz, 1.5 H, CCHCH<sub>3</sub>), 1.47 (d, *J* = 6.9 Hz, 1.5 H, CCHCH<sub>3</sub>), 1.37-1.22 (m, 3 H), 0.93 (d, *J* = 6.3 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>), 0.89 (d, *J* = 6.0 Hz, 3 H, CH(CH<sub>3</sub>)<sub>2</sub>); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 22.3, 21.2.

Example 16

Compound 17: A mixture of compound **19** (0.520 g, 0.573 mmol) and 10% palladium on carbon (0.055 g) in ethanol (10 mL) was stirred under a hydrogen atmosphere (1 atm) for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then mixture was filtered through Celite and concentrated to give a white foam (0.4649 g, 99%). Residue was dissolved in anhydrous DMF (5.0 mL) and to this solution was added phenol (0.097 g, 1.03 mmol), diisopropylethylamine (0.36 mL, 2.06 mmol) followed by benzotriazol-1-yloxytripyrroldinophosphonium hexafluorophosphate (0.536 g, 1.03 mmol). Reaction mixture was stirred for 20 h, then concentrated and residue was dissolved in EtOAc and washed with 1 N HCl, H<sub>2</sub>O, sat. NaHCO<sub>3</sub>, brine and dried (MgSO<sub>4</sub>). Concentration and purification (silica gel, 2 % MeOH/CH<sub>2</sub>Cl<sub>2</sub>) gave a white foam (0.180 g, 35%).

Scheme 4



Reagents and conditions: i. a. 48% HBr, 120°C, 65%; b. H<sub>2</sub>, Pd(OH)<sub>2</sub>, EtOH, 100%;  
 ii. CbzCl, NaOH, toluene/H<sub>2</sub>O, 0°C to rt, 43%; b. 22, CsCO<sub>3</sub>, CH<sub>3</sub>CN, 99%;  
 iii. a. H<sub>2</sub>, Pd/C, AcOH, EtOAc/EtOH, 95%; b. 24, NaBH(OAc)<sub>3</sub>, 1,2-DCE, 21%;  
 iv. 4% HF/CH<sub>3</sub>CN, 62%.

### Example 17

Compound 21: Compound 20 (11.5 g, 48.1 mmol) in 48% HBr (150 mL) was heated at 120°C for 4 h, then cooled to room temperature and diluted with EtOAc. Mixture was neutralized with saturated NaHCO<sub>3</sub> solution and solid NaHCO<sub>3</sub> and extracted with EtOAc containing MeOH. Organic layer dried (MgSO<sub>4</sub>), concentrated, and purified (silica gel, 1:2

EtOAc/Hex with 1% MeOH) to give a brown solid (7.0 g, 65%). The resulting compound (7.0 g, 31.1 mmol) and 10% palladium hydroxide (2.1 g) in EtOH (310 mL) was stirred under a hydrogen atmosphere for 1 d, then filtered through Celite and concentrated to give an off-white solid (4.42 g, 100%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.01 (d, *J* = 7.8 Hz, 1 H, Ar), 6.64 (s, 1 H, Ar), 6.61 (d, *J* = 8.1 Hz, 2 H, Ar), 4.07 (s, 2 H, ArCH<sub>2</sub>N), 4.05 (s, 2 H, ArCH<sub>2</sub>N).

#### Example 18

Compound 22: To a solution of compound **21** (4.42 g, 32.7 mmol) in 1.0 M NaOH (98 mL, 98.25 mmol) at 0°C was added dropwise benzyl chloroformate (7.00 mL, 49.13 mmol) in toluene (7 mL). After addition was complete, reaction mixture was stirred overnight at room temperature. Reaction mixture was diluted with EtOAc and extracted with EtOAc (3x). Combined organic layer was dried (MgSO<sub>4</sub>), concentrated and purified (silica gel, 2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give a white solid (3.786 g, 43%). The resulting compound (0.6546 g, 2.43 mmol) was dissolved in anhydrous acetonitrile (10 mL), and compound **23** (0.782 g, 2.92 mmol) was added, followed by cesium carbonate (1.583 g, 4.86 mmol). Reaction mixture was stirred for 2 h at room temperature, then filtered, concentrated, and purified (3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give a brownish oil (1.01 g, 99%).

#### Example 19

Compound 25: To a solution of compound **22** (0.100 g, 0.238 mmol) in EtOAc/EtOH (2 mL, 1:1) was added acetic acid (14 µL, 0.238 mmol) and 10% palladium on carbon (0.020 g) and the mixture was stirred under a hydrogen atmosphere for 2 h. Celite was added to the reaction mixture and stirred for 5 min, then filtered through Celite. Concentration and drying under high vacuum gave a reddish film (0.0777 g, 95%). The resulting amine (0.0777 g, 0.225 mmol) and aldehyde **24** (0.126 g, 0.205 mmol) in 1,2-dichloroethane (1.2 mL) were stirred for 5 min at 0°C, then sodium triacetoxyborohydride (0.0608 g, 0.287 mmol) was added. Reaction mixture was stirred for 1 h at 0°C, then quenched with saturated NaHCO<sub>3</sub> solution and brine. Extracted with EtOAc, the organic layer was dried (MgSO<sub>4</sub>), concentrated and purified (silica gel, 2% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give a brown foam (38.7 mg, 21%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.74 (d, *J* = 8.7 Hz, 2 H, Ar), 7.09 (d, *J* = 8.7 Hz, 1 H, Ar), 7.05-6.72 (m, 4 H, Ar), 5.71 (d, *J* = 5.1 Hz, 1 H), 5.22-5.07 (m, 2 H), 4.22-4.17 (m, 7 H),

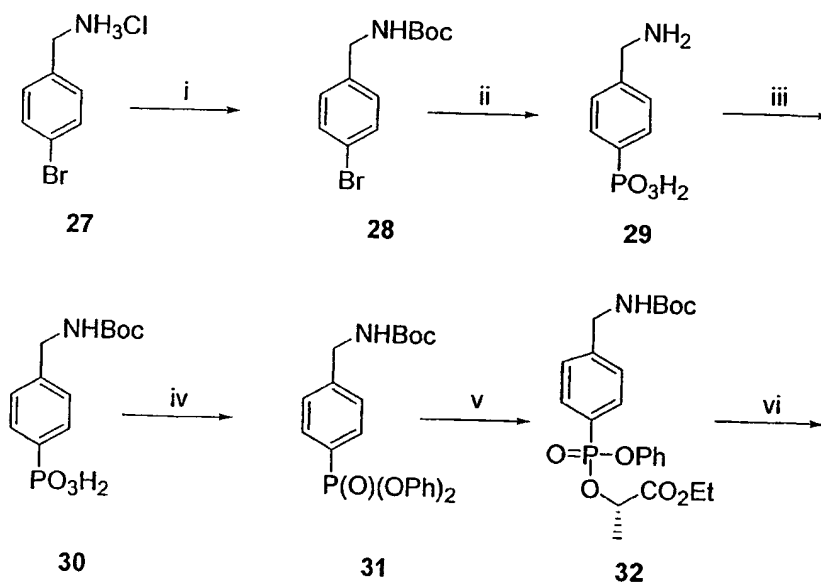


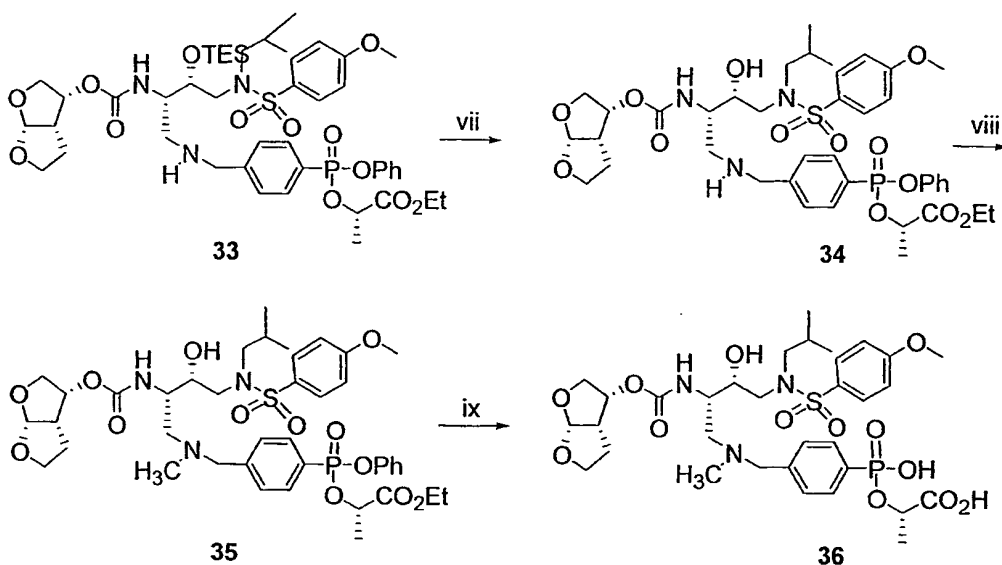
4.16-3.69 (m, 9 H), 3.82 (s, 3 H), 3.25-2.51 (m, 7 H), 2.22-1.70 (m, 3 H), 1.37 (t,  $J = 6.9$  Hz, 6 H), 1.10-0.58 (m, 21 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.5.

### Example 20

Compound 26: To a solution of compound 25 (38.7 mg, 0.0438 mmol) in acetonitrile (0.5 mL) at  $0^\circ\text{C}$  was added 48% HF (0.02 mL). The reaction mixture was stirred at room temperature for 2 h, then quenched with saturated  $\text{NaHCO}_3$  solution and extracted with EtOAc. Organic layer was separated, dried ( $\text{MgSO}_4$ ), concentrated and purified (silica gel, 3 to 5% MeOH/ $\text{CH}_2\text{Cl}_2$ ) to give a red film (21.2 mg, 62%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.73 (d,  $J = 8.7$  Hz, 2 H, Ar), 7.10 (d,  $J = 8.7$  Hz, 1 H, Ar), 6.97 (d,  $J = 8.70$  Hz, 2 H), 6.90-6.76 (m, 2 H), 5.72 (d,  $J = 5.1$  Hz, 1 H), 5.41 (d,  $J = 9.0$  Hz, 1 H), 5.15 (q,  $J = 6.6$  Hz, 1 H), 4.38-4.17 (m, 7 H), 4.16-3.65 (m, 9 H), 3.87 (s, 3 H), 3.20-2.82 (m, 7 H), 2.75-1.79 (m, 3 H), 1.37 (t,  $J = 6.9$  Hz, 6 H), 0.90 (d,  $J = 6.6$  Hz, 3 H), 0.88 (d,  $J = 6.6$  Hz, 3 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  19.3.

### Scheme 5





Reagents and conditions: i.  $\text{Boc}_2\text{O}$ ,  $\text{NaOH}$ ,  $\text{H}_2\text{O}$ , 96%;  
 ii. a.  $\text{HP}(\text{OEt})_2$ ,  $\text{Et}_3\text{N}$ ,  $(\text{PPh}_3)_4\text{Pd}$ ,  $90^\circ\text{C}$ , b.  $\text{TMSBr}$ ,  $\text{CH}_3\text{CN}$ , 65%;  
 iii.  $\text{Boc}_2\text{O}$ ,  $\text{NaOH}$ ,  $\text{THF}/\text{H}_2\text{O}$ , 89%; iv.  $\text{PhOH}$ ,  $\text{DCC}$ ,  $\text{pyr}$ ,  $70^\circ\text{C}$ , 71%;  
 v. a.  $\text{NaOH}$ ,  $\text{CH}_3\text{CN}$ , 94%; b.  $\text{Et lactate}$ ,  $\text{DCC}$ ,  $\text{pyr}$ ,  $70^\circ\text{C}$ , 80%; vi. a.  $\text{TFA}$ ,  $\text{CH}_2\text{Cl}_2$ ;  
 b. **24**,  $\text{AcOH}$ ,  $\text{NaBH}_3\text{CN}$ ,  $\text{EtOH}$ , 33%; vii. 4%  $\text{HF}/\text{CH}_3\text{CN}$ , 88%;  
 viii.  $\text{HCHO}$ ,  $\text{AcOH}$ ,  $\text{NaBH}_3\text{CN}$ ,  $\text{EtOH}$ , 67%;  
 ix.  $\text{CH}_3\text{CN}$ ,  $\text{DMSO}$ ,  $\text{PBS}$ , porcine liver esterase,  $38^\circ\text{C}$ , 21%.

### Example 21

Compound 28: To a mixture of 4-bromobenzylamine hydrochloride (15.23 g, 68.4 mmol) in H<sub>2</sub>O (300 mL) was added sodium hydroxide (8.21 g, 205.2 mmol), followed by di-*tert*-butyl dicarbonate (16.45 g, 75.3 mmol). Reaction mixture was vigorously stirred for 18 h, then diluted with EtOAc (500 mL). Organic layer separated and aqueous layer extracted with EtOAc (200 mL). Combined organic layer was dried (MgSO<sub>4</sub>), concentrated and dried under high vacuum to give a white solid (18.7 g, 96%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.41 (d, *J* = 8.4 Hz, 2 H), 7.12 (d, *J* = 8.3 Hz, 2 H), 4.82 (s, 1 H, *NH*), 4.22 (d, *J* = 6.1 Hz, 2 H), 1.41 (s, 9 H).

### Example 22

Compound 29: Compound 28 (5.00 g, 17.47 mmol) was coevaporated with toluene. Diethyl phosphite (11.3 mL, 87.36 mmol) was added and mixture was coevaporated with toluene

(2x). Triethylamine (24.0 mL, 174.7 mmol) was added and mixture was purged with argon for 10 min, then tetrakis(triphenylphosphine) palladium(0) (4.00 g, 3.49 mmol) was added. Reaction mixture was refluxed for 18 h, cooled, concentrated and diluted with EtOAc. Washed with 0.5 N HCl, 0.5 M NaOH, H<sub>2</sub>O, brine and dried (MgSO<sub>4</sub>). Concentrated and purification (silica gel, 70% EtOAc/Hex) gave an impure reaction product as a yellow oil (6.0 g). This material (6.0 g) was dissolved in anhydrous acetonitrile (30 mL) and cooled to 0°C. Bromotrimethylsilane (11.5 mL, 87.4 mmol) was added and reaction mixture was warmed to room temperature over 15 h. Reaction mixture was concentrated, dissolved in MeOH (50 mL) and stirred for 1.5 h. H<sub>2</sub>O (1 mL) was added and mixture stirred for 2 h. Concentrated to dryness and dried under high vacuum, then triturated with Et<sub>2</sub>O containing 2% MeOH to give a white solid (3.06 g, 65 %). <sup>1</sup>H NMR (300 MHz, D<sub>2</sub>O): δ 7.67 (dd, *J* = 12.9, 7.6 Hz, 2 H), 7.45-7.35 (m, 2 H), 4.10 (s, 2 H); <sup>31</sup>P NMR (121 MHz, D<sub>2</sub>O): δ 12.1.

#### Example 23

Compound 30: Compound **29** (4.78 g, 17.84 mmol) was dissolved in H<sub>2</sub>O (95 mL) containing sodium hydroxide (3.57 g, 89.20 mmol). Di-*tert*-butyl dicarbonate (7.63 g, 34.94 mmol) was added, followed by THF (25 mL). The clear reaction mixture was stirred overnight at room temperature then concentrated to ~100 mL. Washed with EtOAc and acidified to pH 1 with 1 N HCl and extracted with EtOAc (7x). Combined organic layer was dried (MgSO<sub>4</sub>), concentrated and dried under high vacuum. Trituration with Et<sub>2</sub>O gave a white powder (4.56 g, 89%). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): δ 7.85-7.71 (m, 2 H), 7.39-7.30 (m, 2 H), 4.26 (s, 2 H), 1.46 (s, 9 H); <sup>31</sup>P NMR (121 MHz, CD<sub>3</sub>OD): δ 16.3.

#### Example 24

Compound 31: Compound **30** (2.96 g, 10.32 mmol) was coevaporated with anhydrous pyridine (3 x 10 mL). To this residue was added phenol (9.71 g, 103.2 mmol) and mixture was coevaporated with anhydrous pyridine (2 x 10 mL). Pyridine (50 mL) was added and solution heated to 70°C. After 5 min, 1,3-dicyclohexylcarbodiimide (8.51 g, 41.26 mmol) was added and resulting mixture was stirred for 8 h at 70°C. Reaction mixture was cooled and concentrated and coevaporated with toluene. Residue obtained was diluted with EtOAc and the resulting precipitate was removed by filtration. The filtrate was concentrated and purified (silica gel, 20 to 40% EtOAc/Hex, another column 30 to 40% EtOAc/Hex) to give a

white solid (3.20 g, 71%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.90 (dd,  $J = 13.8, 8.2$  Hz, 2 H), 7.41-7.10 (m, 14 H), 5.17 (br s, 1 H, *NH*), 4.35 (d,  $J = 5.2$  Hz, 2 H), 1.46 (s, 9 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  11.8.

#### Example 25

Compound 32: To a solution of compound **31** (3.73 g, 8.49 mmol) in acetonitrile (85 mL) at  $0^\circ\text{C}$  was added 1 M NaOH (21.2 mL, 21.21 mmol). Reaction mixture was stirred at  $0^\circ\text{C}$  for 30 min, then warmed to room temperature over 4 h. Reaction mixture cooled to  $0^\circ\text{C}$  and Dowex ( $\text{H}^+$ ) residue was added to pH 2. Mixture was filtered, concentrated and residue obtained was triturated with EtOAc/Hex (1:2) to give a white powder (2.889 g, 94%). This compound (2.00 g, 5.50 mmol) was coevaporated with anhydrous pyridine ( $3 \times 10$  mL). The residue was dissolved in anhydrous pyridine (30 mL) and ethyl (*S*)-lactate (6.24 mL, 55 mmol) and reaction mixture was heated to  $70^\circ\text{C}$ . After 5 min, 1,3-dicyclocarbodiimide (4.54 g, 22.0 mmol) was added. Reaction mixture was stirred at  $70^\circ\text{C}$  for 5 h, then cooled and concentrated. Residue was dissolved in EtOAc and precipitate was removed by filtration. The filtrate was concentrated and purified (25 to 35% EtOAc/Hex, another column 40% EtOAc/Hex) to give a colorless oil (2.02 g, 80%).  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  7.96-7.85 (m, 2 H), 7.42-7.35 (m, 2 H), 7.35-7.08 (m, 4 H), 5.16-5.00 (m, 1 H), 4.93 (s, 1 H, *NH*), 4.37 (d,  $J = 5.5$  Hz, 1 H), 4.21 (q,  $J = 7.3$  Hz, 1 H), 4.11 (dq,  $J = 5.7, 2.2$  Hz, 1 H), 1.62-1.47 (m, 3 H), 1.47 (s, 9 H), 1.27 (t,  $J = 7.3$  Hz, 1.5 H), 1.17 (t,  $J = 7.3$  Hz, 1.5 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.1, 15.0.

#### Example 26

Compound 33: Compound **32** (2.02 g, 4.36 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (41 mL) and cooled to  $0^\circ\text{C}$ . To this solution was added trifluoroacetic acid (3.5 mL) and reaction mixture was stirred at  $0^\circ\text{C}$  for 1 h, then at room temperature for 3 h. Reaction mixture was concentrated, coevaporated with EtOAc and diluted with  $\text{H}_2\text{O}$  (400 mL). Mixture was neutralized with Amberlite IRA-67 weakly basic resin, then filtered and concentrated. Coevaporation with MeOH and dried under high vacuum to give the TFA amine salt as a semi-solid (1.48 g, 94%). To a solution of the amine (1.48 g, 4.07 mmol) in absolute ethanol (20 mL) at  $0^\circ\text{C}$  was added aldehyde **24** (1.39 g, 2.26 mmol), followed by acetic acid (0.14 mL, 2.49 mmol). After stirring for 5 min, sodium cyanoborohydride (0.284 g, 4.52 mmol)

was added and reaction mixture stirred for 30 min at 0°C. Reaction was quenched with saturated NaHCO<sub>3</sub> solution and diluted with EtOAc and H<sub>2</sub>O. Aqueous layer was extracted with EtOAc (3x) and combined organic layer was dried (MgSO<sub>4</sub>), concentrated and purified (silica gel, 2 to 4% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give white foam (0.727 g, 33%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.98-7.86 (m, 2 H), 7.71 (d, *J* = 8.6 Hz, 2 H), 7.49 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.98 (d, *J* = 8.8 Hz, 2 H), 5.72 (d, *J* = 5.1 Hz, 1 H), 5.28-5.00 (m, 2 H), 4.30-3.72 (m, 12 H), 3.42-3.58 (m, 1 H), 3.20-2.68 (m, 7 H), 2.25-1.42 (m, 6 H), 1.26 (t, *J* = 7.2 Hz, 1.5 H), 1.17 (t, *J* = 7.2 Hz, 1.5 H), 1.08-0.50 (m, 21 H); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 16.1, 15.1.

#### Example 27

Compound 34: To a solution of compound 33 (0.727 g, 0.756 mmol) in acetonitrile (7.6 mL) at 0°C was added 48% hydrofluoric acid (0.152 mL) and reaction mixture was stirred for 40 min at 0°C, then diluted with EtOAc and H<sub>2</sub>O. Saturated NaHCO<sub>3</sub> was added and aqueous layer was extracted with EtOAc (2x). Combined organic layer was dried (MgSO<sub>4</sub>), concentrated and purified (silica gel, 4 to 5% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give a colorless foam (0.5655 g, 88%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.95-7.82 (m, 2 H), 7.67 (d, *J* = 8.1 Hz, 2 H), 7.41 (br s, 2 H), 7.38-7.05 (m, 5 H), 6.95 (d, *J* = 7.2 Hz, 2 H), 5.76 (d, *J* = 7.9 Hz, 1 H), 5.67 (d, *J* = 5.0 Hz, 1 H), 5.32-4.98 (m, 2 H), 4.25-3.75 (m, 13 H), 3.25-2.70 (m, 7 H), 2.15-1.76 (m, 3 H), 1.53-1.41 (m, 3 H), 1.25-1.08 (m, 3 H), 0.87 (d, *J* = 4.2 Hz, 6 H); <sup>31</sup>P NMR (121 MHz, CDCl<sub>3</sub>): δ 16.1, 15.0.

#### Example 28

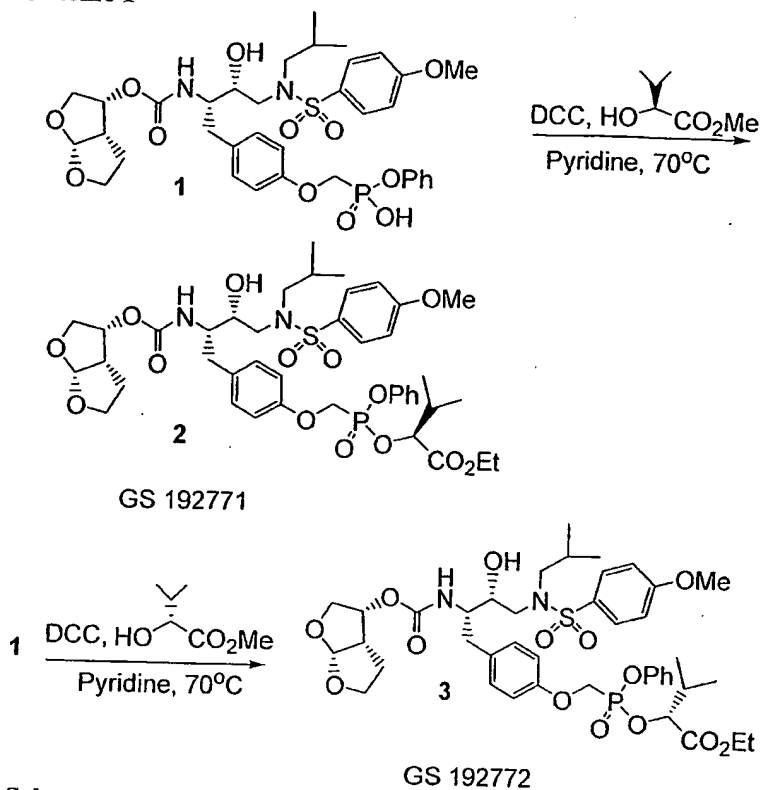
Compound 35: To a solution of compound 33 (0.560 g, 0.660 mmol) in absolute ethanol (13 mL) at 0°C was added 37% formaldehyde (0.54 mL, 6.60 mmol), followed by acetic acid (0.378 mL, 6.60 mmol). The reaction mixture was stirred at 0°C for 5 min, then sodium cyanoborohydride (0.415 g, 6.60 mmol) was added. Reaction mixture was warmed to room temperature over 2 h, then quenched with saturated NaHCO<sub>3</sub> solution. EtOAc was added and mixture was washed with brine. Aqueous layer was extracted with EtOAc (2x) and combined organic layer was dried (MgSO<sub>4</sub>), concentrated and purified (silica gel, 3% MeOH/CH<sub>2</sub>Cl<sub>2</sub>) to give a white foam (0.384 g, 67%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 7.95-7.82 (m, 2 H), 7.71 (d, *J* = 8.4 Hz, 2 H), 7.38 (br s, 2 H), 7.34-7.10 (m, 5 H), 6.98 (d, *J* = 8.8 Hz, 2 H), 5.72 (d, *J* = 5.0 Hz, 1 H), 5.50 (br s, 1 H), 5.19-5.01 (m, 2 H), 4.29-3.75 (m, 10 H),

3.85 (s, 3 H), 3.35-2.70 (m, 7 H), 2.23 (s, 3 H), 2.17-1.79 (m, 3 H), 1.54 (d,  $J = 6.9$  Hz, 1.5 H), 1.48 (d,  $J = 6.8$  Hz, 1.5 H), 1.25 (t,  $J = 7.2$  Hz, 1.5 H), 1.16 (t,  $J = 7.2$  Hz, 1.5 H), 0.92 (d,  $J = 6.6$  Hz, 3 H), 0.87 (d,  $J = 6.6$  Hz, 3 H).  $^{31}\text{P}$  NMR (121 MHz,  $\text{CDCl}_3$ ):  $\delta$  16.0, 14.8.

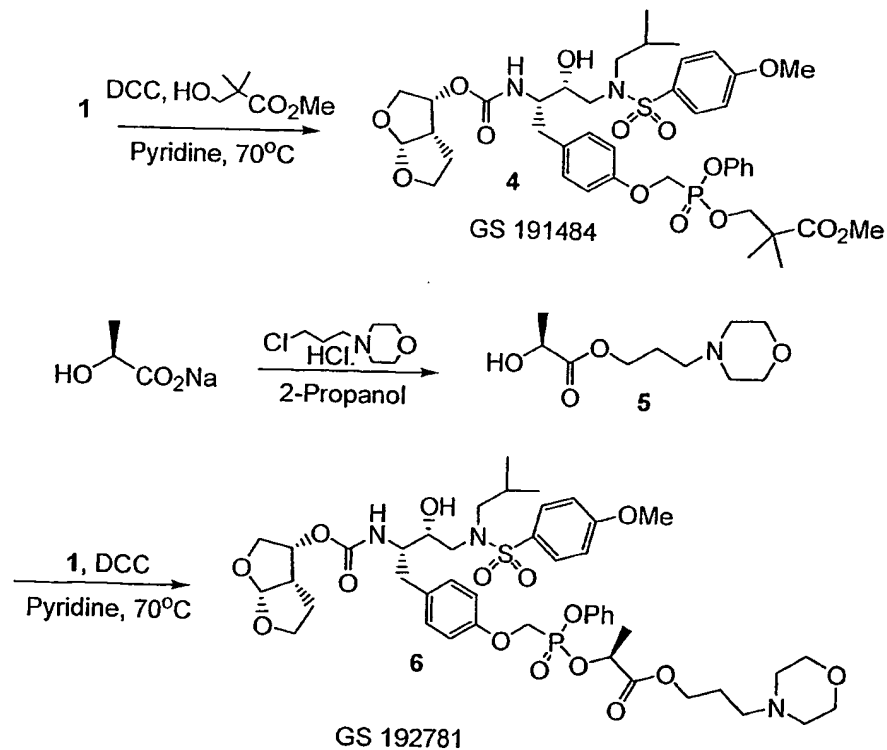
#### Example 29

Compound 36: To a solution of compound 35 (44 mg, 0.045 mmol) in acetonitrile (1.0 mL) and DMSO (0.5 mL) was added phosphate buffered saline (pH 7.4, 5.0 mL) to give a cloudy white suspension. Porcine liver esterase (200  $\mu\text{L}$ ) was added and reaction mixture was stirred for 48 h at 38°C. Additional esterase (600  $\mu\text{L}$ ) was added and reaction was continued for 4 d. Reaction mixture was concentrated, diluted with MeOH and the resulting precipitate removed by filtration. Filtrate was concentrated and purified by reverse phase HPLC to give a white powder after lyophilization (7.2 mg, 21%).  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  7.95 (br s, 2 H), 7.76 (d,  $J = 8.4$  Hz, 2 H), 7.64 (br s, 2 H), 7.13 (d,  $J = 8.7$  Hz, 2 H), 5.68 (d,  $J = 5.1$  Hz, 1 H), 5.14 (br s, 1 H), 4.77 (br s, 1 H), 4.35-3.59 (m, 8 H), 3.89 (s, 3 H), 3.45-2.62 (m, 10 H), 2.36-1.86 (m, 3 H), 1.44 (d,  $J = 6.3$  Hz, 3 H), 0.92 (d,  $J = 6.6$  Hz, 3 H), 0.84 (d,  $J = 6.6$  Hz, 3 H);  $^{31}\text{P}$  NMR (121 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  13.8.

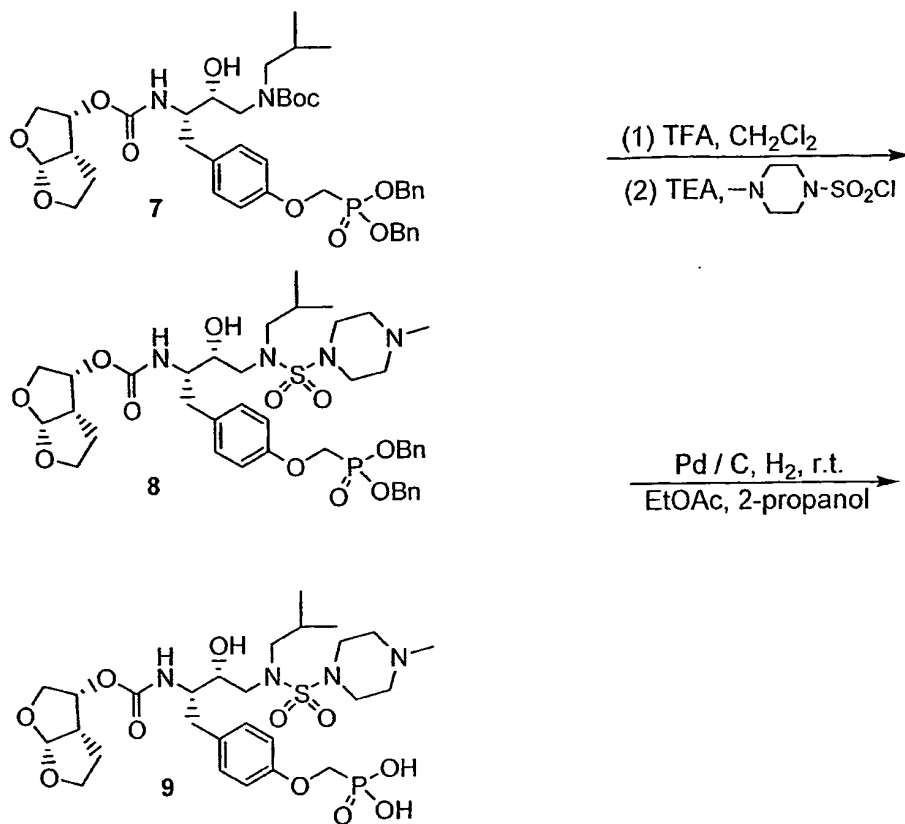
## Scheme 1



## Scheme 2

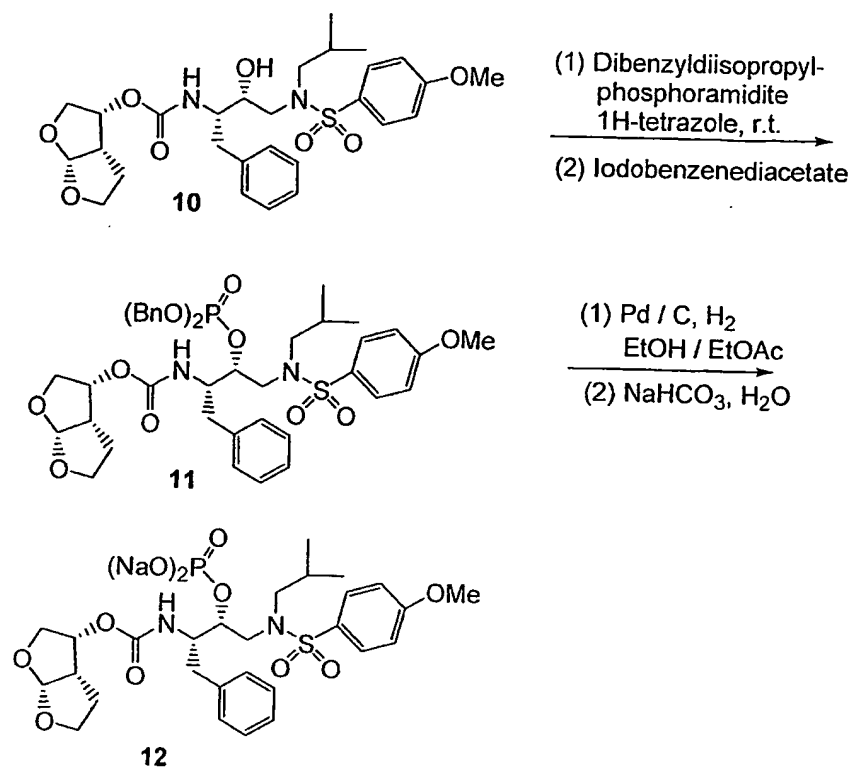


Scheme 3

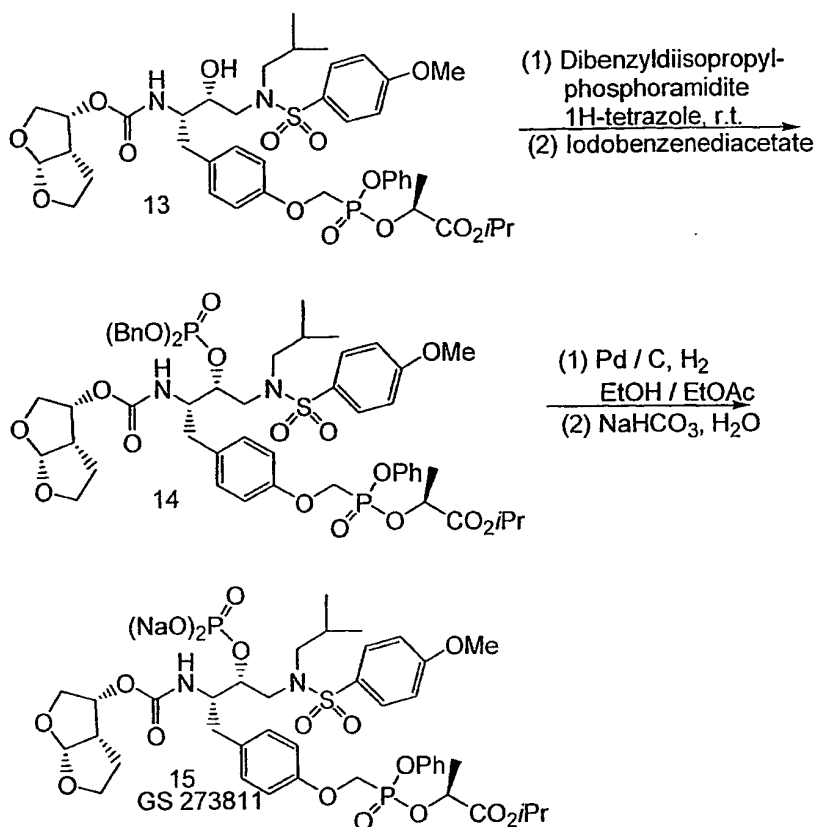




Scheme 4



## Scheme 5



## Example 1

Monophospholactate 2: A solution of 1 (0.11 g, 0.15 mmol) and  $\alpha$ -hydroxyisovaleric acid ethyl-(S)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl, H<sub>2</sub>O, saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the monophospholactate (35 mg, 28%, GS 192771, 1/1 diastereomeric mixture) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  7.71 (d, J = 8.7 Hz, 2H), 7.36-7.14 (m, 7H), 6.99 (d, J = 8.7 Hz, 2H), 6.94-6.84 (dd, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.00-4.85 (m, 3H), 4.55 (dd, 1H), 4.41 (dd, 1H), 4.22-4.07 (m, 2H), 3.96-3.68 (m, 9H), 3.12-2.74 (m, 7H), 2.29 (m, 1H), 1.85-1.57

(m, 3H), 1.24 (m, 3H), 1.05 (d,  $J = 6.6$  Hz, 3H), 0.98 (d,  $J = 6.6$  Hz, 3H), 0.9 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.7, 15.1.

### Example 2

Monophospholactate 3: A solution of 1 (0.11 g, 0.15 mmol) and  $\alpha$ -hydroxyisovaleric acid ethyl-(R)-ester (71 mg, 0.49 mmol) in pyridine (2 mL) was heated to  $70^\circ\text{C}$  and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at  $70^\circ\text{C}$  for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl,  $\text{H}_2\text{O}$ , saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (35 mg, 28%, GS 192772, 1/1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d,  $J = 8.7$  Hz, 2H), 7.35-7.13 (m, 7H), 6.98 (d,  $J = 8.7$  Hz, 2H), 6.93-6.83 (dd, 2H), 5.64 (d,  $J = 5.4$  Hz, 1H), 5.04-4.85 (m, 3H), 4.54 (dd, 1H), 4.39 (dd, 1H), 4.21-4.06 (m, 2H), 3.97-3.67 (m, 9H), 3.12-2.75 (m, 7H), 2.27 (m, 1H), 1.83-1.57 (m, 3H), 1.26 (m, 3H), 1.05 (d,  $J = 6.6$  Hz, 3H), 0.98 (d,  $J = 6.6$  Hz, 3H), 0.9 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.7, 15.1.

### Example 3

Monophospholactate 4: A solution of 1 (0.10 g, 0.13 mmol) and methyl-2,2-dimethyl-3-hydroxypropionate (56  $\mu\text{L}$ , 0.44 mmol) in pyridine (1 mL) was heated to  $70^\circ\text{C}$  and 1,3-dicyclohexylcarbodiimide (91 mg, 0.44 mmol) was added. The reaction mixture was stirred at  $70^\circ\text{C}$  for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and 0.2 N HCl. The EtOAc layer was washed with 0.2 N HCl,  $\text{H}_2\text{O}$ , saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (72 mg, 62%, GS 191484) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d,  $J = 8.7$  Hz, 2H), 7.34 (m, 2H), 7.25-7.14 (m, 5H), 7.00 (d,  $J = 9.0$  Hz, 2H), 6.87 (d,  $J = 8.7$  Hz, 2H), 5.65 (d,  $J = 5.4$  Hz, 1H), 5.05 (m, 2H), 4.38 (d,  $J = 9.6$  Hz, 2H),

4.32-4.20 (m, 2H), 4.00 (m, 2H), 3.87-3.63 (m, 12H), 3.12-2.78 (m, 7H), 1.85-1.67 (m, 3H), 1.20 (m, 6H), 0.91 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.0.

#### Example 4

Lactate 5: To a suspension of lactic acid sodium salt (5 g, 44.6 mmol) in 2-propanol (60 mL) was added 4-(3-chloropropyl)morpholine hydrochloride (8.30 g, 44.6 mmol). The reaction mixture was heated to reflux for 18 h and cooled to room temperature. The solid was filtered and the filtrate was recrystallized from EtOAc / hexane to give the lactate (1.2 g, 12%).

#### Example 5

Monophospholactate 6: A solution of 1 (0.10 g, 0.13 mmol) and lactate 5 (0.10 g, 0.48 mmol) in pyridine (2 mL) was heated to 70°C and 1,3-dicyclohexylcarbodiimide (0.10 g, 0.49 mmol) was added. The reaction mixture was stirred at 70°C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was suspended in EtOAc and 1,3-dicyclohexyl urea was filtered off. The product was partitioned between EtOAc and  $\text{H}_2\text{O}$ . The EtOAc layer was washed with saturated NaCl, dried with  $\text{Na}_2\text{SO}_4$ , filtered, and concentrated. The crude product was purified by column chromatography on silica gel (4% 2-propanol/ $\text{CH}_2\text{Cl}_2$ ) to give the monophospholactate (30 mg, 24%, GS 192781, 1/1 diastereomeric mixture) as a white solid:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  7.71 (d, J = 8.7 Hz, 2H), 7.38-7.15 (m, 7H), 7.00 (d, J = 8.7 Hz, 2H), 6.91 (m, 2H), 5.65 (d, J = 3.3 Hz, 1H), 5.18-4.98 (m, 3H), 4.54 (dd, 1H), 4.42 (dd, 1H), 4.2 (m, 2H), 4.00-3.67 (m, 16H), 3.13-2.77 (m, 7H), 2.4 (m, 5H), 1.85-1.5 (m, 5H), 1.25 (m, 2H), 0.93 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ )  $\delta$  17.4, 15.4.

#### Example 6

Sulfonamide 8: A solution of dibenzylphosphonate 7 (0.1 g, 0.13 mmol) in  $\text{CH}_2\text{Cl}_2$  (0.5 mL) at 0°C was treated with trifluoroacetic acid (0.25 mL). The solution was stirred for 30 min at 0°C and then warmed to room temperature for an additional 30 min. The reaction mixture was diluted with toluene and concentrated under reduced pressure. The residue was co-evaporated with toluene (2 x), chloroform (2 x), and dried under vacuum to give the ammonium triflate salt which was dissolved in  $\text{CH}_2\text{Cl}_2$  (1 mL) and cooled to 0°C. Triethylamine (72  $\mu\text{L}$ , 0.52 mmol) was added followed by the treatment of 4-methylpiperazinylsulfonyl chloride (25 mg, 0.13 mmol). The solution was stirred for 1 h at

0°C and the product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O. The organic phase was washed with saturated NaCl, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (5% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the sulfonamide 8 (32 mg, 30%, GS 273835) as a white solid: <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 7.35 (m, 10H), 7.11 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 5.65 (d, J = 5.4 Hz, 1H), 5.2-4.91 (m, 4H), 4.2 (d, J = 10.2 Hz, 2H), 4.0-3.69 (m, 6H), 3.4-3.19 (m, 5H), 3.07-2.75 (m, 5H), 2.45 (m, 4H), 2.3 (s, 3H), 1.89-1.44 (m, 7H), 0.93 (m, 6H); <sup>31</sup>P NMR (CDCl<sub>3</sub>) δ 20.3.

#### Example 7

Phosphonic Acid 9: To a solution of 8 (20 mg, 0.02 mmol) in EtOAc (2 mL) and 2-propanol (0.2 mL) was added 10% Pd/C (5 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature overnight. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid (10 mg, 64%) as a white solid.

#### Example 8

Dibenzylphosphonate 11: A solution of 10 (85 mg, 0.15 mmol) and 1*H*-tetrazole (14 mg, 0.20 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) was treated with Dibenzyl-diisopropylphosphoramidite (60 μL, 0.20 mmol) and stirred at room temperature overnight. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (85 mg, 0.11 mmol) which was dissolved in CH<sub>3</sub>CN (2 mL) and treated with iodobenzenediacetate (51 mg, 0.16 mmol). The reaction mixture was stirred at room temperature for 3 h and concentrated. The residue was partitioned between EtOAc and NaHCO<sub>3</sub>. The organic layer was washed with H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the dibenzylphosphonate (45 mg, 52%) as a white solid.

#### Example 9

Disodium Salt of Phosphonic Acid 12: To a solution of 11 (25 mg, 0.03 mmol) in EtOAc (2 mL) was added 10% Pd/C (10 mg). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of

celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid which was dissolved in H<sub>2</sub>O (1 mL) and treated with NaHCO<sub>3</sub> (2.53 mg, 0.06 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (19.77 mg, 95%, GS 273777) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 8.1 Hz, 2H), 7.27-7.09 (m, 5H), 5.57 (d, J = 5.1 Hz, 1H), 5.07 (m, 1H), 4.87-4.40 (m, 3H), 3.93-3.62 (m, 6H), 3.45-2.6 (m, 6H), 2.0 (m, 2H), 1.55 (m, 1H), 0.95-0.84 (m, 6H).

#### Example 10

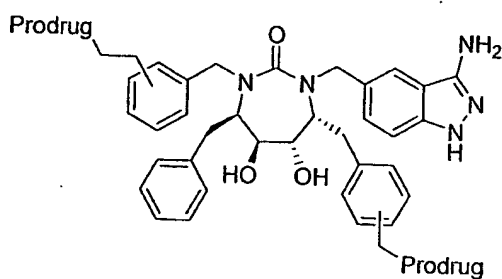
Dibenzylphosphonate 14: A solution of 13 (0.80 g, 0.93 mmol) and 1*H*-tetrazole (98 mg, 1.39 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) was treated with dibenzyl-diisopropylphosphoramidite (0.43 mL, 1.39 mmol) and stirred at room temperature overnight. The product was partitioned between CH<sub>2</sub>Cl<sub>2</sub> and H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. The crude product was purified by column chromatography to give the intermediate dibenzylphosphite (0.68 g, 67%). To a solution of the dibenzylphosphite (0.39 g, 0.35 mmol) in CH<sub>3</sub>CN (5 mL) was added iodobenzenediacetate (0.17 g, 0.53 mmol). The reaction mixture was stirred at room temperature for 2 h and concentrated. The residue was partitioned between EtOAc and NaHCO<sub>3</sub>. The organic layer was washed with H<sub>2</sub>O, dried with Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (3% 2-propanol/CH<sub>2</sub>Cl<sub>2</sub>) to give the dibenzylphosphonate (0.35 g, 88%) as a white solid.

#### Example 11

Disodium Salt of Phosphonic Acid 15: To a solution of 14 (0.39 g, 0.35 mmol) in EtOAc (30 mL) was added 10% Pd/C (0.10 g). The suspension was stirred under H<sub>2</sub> atmosphere (balloon) at room temperature for 4 h. The reaction mixture was filtered through a plug of celite. The filtrate was concentrated and dried under vacuum to give the phosphonic acid, which was dissolved in H<sub>2</sub>O (3 mL) and treated with NaHCO<sub>3</sub> (58 mg, 0.70 mmol). The reaction mixture was stirred at room temperature for 1 h and lyophilized overnight to give the disodium salt of phosphonic acid (0.31 g, 90%, GS 273811) as a white solid: <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 7.81 (d, J = 9.0 Hz, 2H), 7.43-7.2 (m, 7H), 7.13 (d, J = 9.0 Hz, 2H), 6.9 (m, 2H), 5.55 (d, J = 4.8 Hz, 1H), 5.07 (m, 2H), 4.87 (m, 1H), 4.64-4.4 (m, 4H), 3.93-3.62 (m, 9H), 3.33-2.63 (m, 5H), 2.11 (m, 1H), 1.6-1.42 (m, 4H), 1.38-1.25 (m, 7H), 0.95 (d, J = 6.3 Hz, 3H), 0.84 (d, J = 6.3 Hz, 3H).

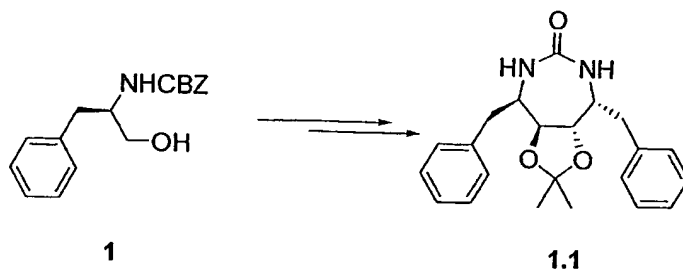
**Examples For The Preparation Of Cyclic Carbonyl-Like Phosphonate Protease Inhibitors (CCPPI)**

**Phosphoramidate Prodrugs**



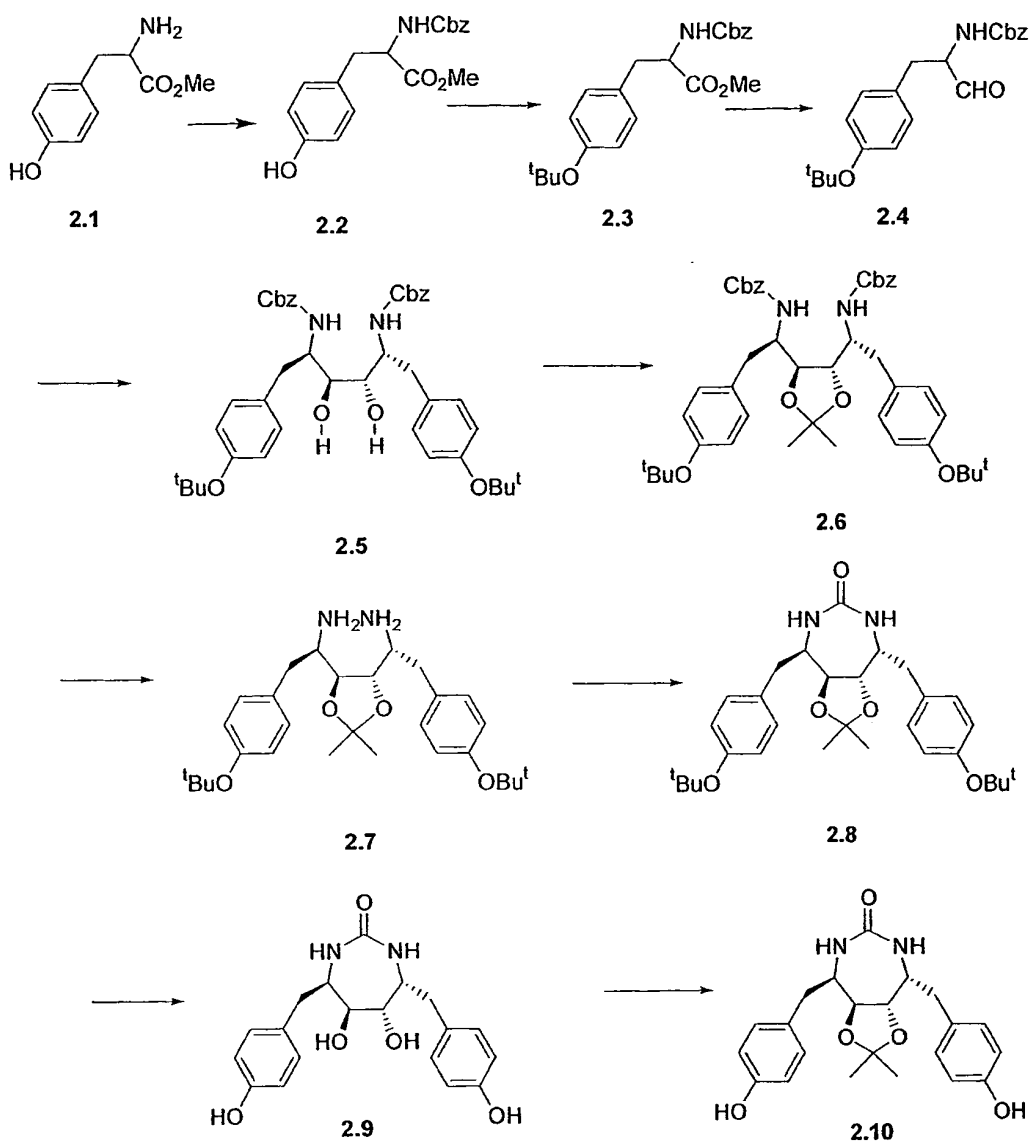
|              |                               |
|--------------|-------------------------------|
| Scheme 1-2   | Scaffold Synthesis            |
| Scheme 3-10  | P2'-Benzyl ether phosphonates |
| Scheme 11-13 | P2'-Alkyl ether phosphonates  |
| Scheme 14-17 | P2'-Benzyl Amide phosphonates |
| Scheme 18-25 | P1-Phosphonates               |
| Scheme 50    | Reagents                      |

**Scheme 1**



The conversion of **1** to **1.1** is described in J. Org Chem 1996, 61, p444-450

## Scheme 2

**2-Benzylloxycarbonylamino-3-(4-tert-butoxy-phenyl)-propionic acid methyl ester (2.3)**

H-D-Tyr-O-me hydrochloride 2.1 (25 g, 107.7 mmol) is dissolved in methylene chloride (150 mL) and aqueous sodium bicarbonate (22 g in 150 mL water), and then cooled to 0°C. To this resulting solution benzyl chloroformate (20 g, 118 mmol) is slowly added. After complete addition, the resulting solution is warmed to room temperature, and is then stirred for 2 h. The organic phase is separated, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under reduced pressure, to give the crude carbamate 2.2 (35g). The crude CBZ-Tyr-OMe product is



dissolved in methylene chloride (300 mL) containing concentrated  $\text{H}_2\text{SO}_4$ . Isobutene is bubbled through the solution for 6 h. The reaction is then cooled to  $0^\circ\text{C}$ , and neutralized with saturated  $\text{NaHCO}_3$  aqueous solution. The organic phase is separated, dried, concentrated under reduced pressure, and purified by silica gel column chromatography to afford the tert-butyl ether 2.3 (25.7 g, 62 %).

**[2-(4-tert-Butoxy-phenyl)-1-formyl-ethyl]-carbamic acid benzyl ester (2.4)**

(Reference J. O. C. 1997, 62, 3884).

To a stirred  $-78^\circ\text{C}$  methylene chloride solution (60 mL) of 2.3, DIBAL (82 mL of 1.5 M in toluene, 123 mmol) was added over 15 min. The resultant solution was stirred at  $-78^\circ\text{C}$  for 30 min. Subsequently, a solution of EtOH/36 % HCl (9/1; 15 mL) is added slowly. The solution is added to a vigorously stirred aqueous HCl solution (600 mL, 1N) at  $0^\circ\text{C}$ . The layers are then separated, and the aqueous phase is extracted with cold methylene chloride. The combined organic phases are washed with cold 1N HCl aqueous solution, water, dried over  $\text{Na}_2\text{SO}_4$ , and then concentrated under reduced pressure to give the crude aldehyde 2.4 (20 g, 91 %).

**[4-Benzyloxycarbonylamino-1-(4-tert-butoxy-benzyl)-5-(4-tert-butoxy-phenyl)-2,3-dihydroxy-pentyl]-carbamic acid benzyl ester (2.5)**

To a slurry of  $\text{VCl}_3(\text{THF})_3$  in methylene chloride (150 mL) at room temperature is added Zinc powder (2.9 g, 44 mmol), and the resulting solution is then stirred at room temperature for 1 hour. A solution of aldehyde 2.4 (20 g, 56 mmol) in methylene chloride (100 mL) is then added over 10 min. The resulting solution is then stirred at room temperature overnight, poured into an ice-cold  $\text{H}_2\text{SO}_4$  aqueous solution (8 mL in 200 mL), and stirred at  $0^\circ\text{C}$  for 30 min. The methylene chloride solution is separated, washed with 1N HCl until the washing solution is light blue. The organic solution is then concentrated under reduced pressure (solids are formed during concentration), and diluted with hexane. The precipitate is collected and washed thoroughly with a hexane/methylene chloride mixture to give the diol product 2.5. The filtrate is concentrated under reduced pressure and subjected to silica gel chromatography to afford a further 1.5 g of 2.5. (Total = 13 g, 65 %).

**[1-{5-[1-Benzyloxycarbonylamino-2-(4-tert-butoxy-phenyl)-ethyl]-2,2-dimethyl-[1,3]dioxolan-4-yl}-2-(4-tert-butoxy-phenyl)-ethyl]-carbamic acid benzyl ester (2.6)**

Diol 2.5 (5 g, 7 mmol) is dissolved in acetone (120 mL), 2,2-dimethoxypropane (20 mL), and pyridinium p-toluenesulfonate (120 mg, 0.5 mmol). The resulting solution is refluxed for 30 min., and then concentrated under reduced pressure to almost dryness. The resulting mixture is partitioned between methylene chloride and saturated NaHCO<sub>3</sub> aqueous solution, dried, concentrated under reduced pressure, and purified by silica gel column chromatography to afford isopropylidene protected diol 2.6 (4.8 g, 92 %).

**4,8-Bis-(4-tert-butoxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxo-5,7-diaza-azulen-6-one(2.8)**

The diol 2.6 is dissolved in EtOAc/EtOH (10 mL/2 mL) in the presence of 10 % Pd/C and hydrogenated at atmospheric pressure to afford the diamino compound 2.7. To a solution of crude 2.7 in 1,1,2,2-tetrachloroethane is added 1,1-carboxydiimidazole (1.05 g, 6.5 mmol) at room temperature. The mixture is stirred for 10 min, and the resulting solution is then added dropwise to a refluxing 1,1',2,2'-tetrachloroethane solution (150 mL). After 30 min., the reaction mixture is cooled to room temperature, and washed with 5 % citric acid aqueous solution, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure, and purified by silica gel column chromatography to afford the cyclourea derivative 2.8 (1.92 g, 60 % over 2 steps).

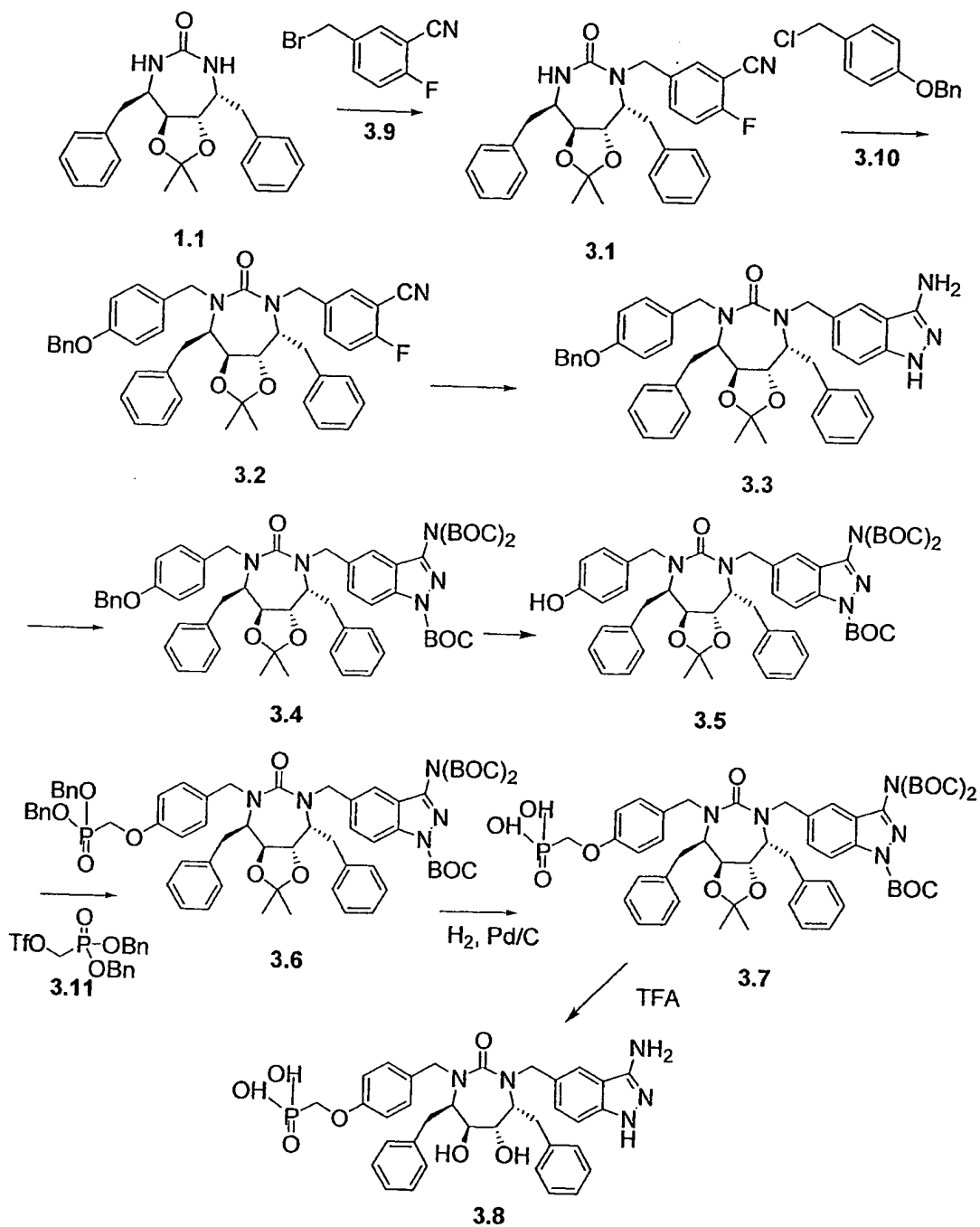
**5,6-Dihydroxy-4,7-bis-(4-hydroxy-benzyl)-[1,3]diazepan-2-one (2.9)**

Cyclic Urea 2.8 (0.4 g, 0.78 mmol) was dissolved in dichloromethane (3 mL) and treated with TFA (1 mL). The mixture was stirred at room temperature for 2 h upon which time a white solid precipitated. 2 drops of water and methanol (2 mL) were added and the homogeneous solution was stirred for 1 h and concentrated under reduced pressure. The crude solid, 2.9, was dried overnight and then used without further purification.

**4,8-Bis-(4-hydroxy-benzyl)-2,2-dimethyl-hexahydro-1,3-dioxo-5,7-diaza-azulen-6-one (2.10)**

Diol 2.9 (1.8 g, 5.03 mmol) was dissolved in DMF (6 mL) and 2,2-dimethoxypropane (12 mL). P-TsOH (95 mg) was added and the mixture stirred at 65°C for 3 h. A vacuum was applied to remove water and then the mixture was stirred at 65°C for a further 1 h. The excess dimethoxypropane was then distilled and the remaining DMF solution was then

allowed to cool. The solution of acetone 2.10 can then be used without further purification in future reactions.

**Scheme 3**

**3-Cyano-4-fluorobenzyl urea 3.1** : A solution of urea 1.1 (1.6 g, 4.3 mmol) in THF was treated with sodium hydride (0.5 g of 60 % oil dispersion, 13 mmol). The mixture was stirred at room temperature for 30 min and then treated with 3-cyano-4-fluorobenzyl bromide 3.9 (1.0 g, 4.8 mmol). The resultant solution was stirred at room temperature for 3 h, concentrated under reduced pressure, and then partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine solution containing 1 % citric acid. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 15-25% ethyl acetate in hexanes to yield urea 3.1 (1.5 g, 69 %) as a white form.

**Benzyl ether 3.2** : A solution of 3.1 (0.56 g, 1.1 mmol) in DMF (5 mL) was treated with sodium hydride (90 mg of 60 % oil dispersion, 2.2 mmol) and the resultant mixture stirred at room temperature for 30 min. 4-Benzyloxy benzyl chloride 3.10 (0.31 g, 1.3 mmol) was added and the resultant solution stirred at room temperature for 3 h. The mixture was concentrated under reduced pressure and then partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine solution. The organic phase was separated, dried over sodium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by silica gel eluting with 1-10% ethyl acetate in hexanes to yield compound 3.2 (0.52 g, 67 %) as white form.

**Indazole 3.3**: Benzyl ether 3.2 (0.51 g, 0.73 mmol) was dissolved in n-butanol (10 mL) and treated with hydrazine hydrate (1 g, 20 mmol). The mixture was refluxed for 4 h and then allowed to cool to room temperature. The mixture was concentrated under reduced pressure and the residue was then partitioned between  $\text{CH}_2\text{Cl}_2$  and 10 % citric acid solution. The organic phase was separated, concentrated under reduced pressure, and then purified by silica gel column eluting with 5% methanol in  $\text{CH}_2\text{Cl}_2$  to afford indazole 3.3 (0.42 g, 82 %) as white solid.

**Boc-indazole 3.4** : A solution of indazole 3.3 (0.4 g, 0.59 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was treated with diisopropylethylamine (0.19 g, 1.5 mmol), DMAP (0.18 g, 1.4 mmol), and di-tert-butyl dicarbonate (0.4 g, 2 mmol). The mixture was stirred at room temperature for 3 h and then partitioned between  $\text{CH}_2\text{Cl}_2$  and 5 % citric acid solution. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The

residue was purified by silica gel eluting with 2% methanol in  $\text{CH}_2\text{Cl}_2$  to afford **3.4** (0.42 g, 71 %).

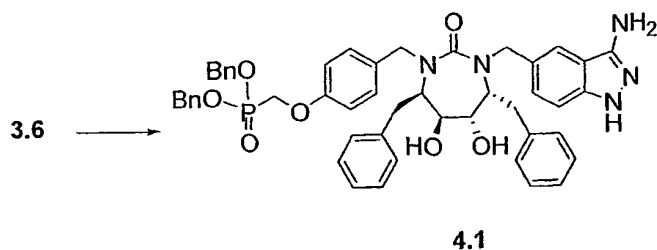
**Phenol 3.5** : A solution of **3.4** (300 mg, 0.3 mmol) in ethyl acetate (10 mL) and methanol (10 mL) was treated with 10 % Pd/C (40 mg) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **3.5** as a white powder. This was used without further purification.

**Dibenzyl ester 3.6** : A solution of **3.5** (0.1 mmol) in THF (5 mL) was treated with dibenzyl triflate **3.11** (90 mg, 0.2 mmol), and cesium carbonate (0.19 g, 0.3 mmol). The mixture was stirred at room temperature for 4 h and then concentrated under reduced pressure. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 20-40% ethyl acetate in hexanes to afford **3.6** (70 mg, 59 %).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.07 (d, 1H), 7.20-7.43 (m, 16H), 7.02-7.15 (m, 8 H), 6.80 (d, 2H), 5.07-5.18 (m, 4H), 5.03 (d, 1H), 4.90 (d, 1H), 4.20 (d, 2H), 3.74-3.78 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H) 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.26 (s, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 20.5 ppm.

**Phosphonic acid 3.7**: A solution of dibenzylphosphonate **3.6** (30 mg) in EtOAc (10 mL) was treated with 10% Pd/C (10 mg) and the mixture was stirred under a hydrogen atmosphere (balloon) for 3 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford phosphonic acid **3.7**. This was used without further purification.

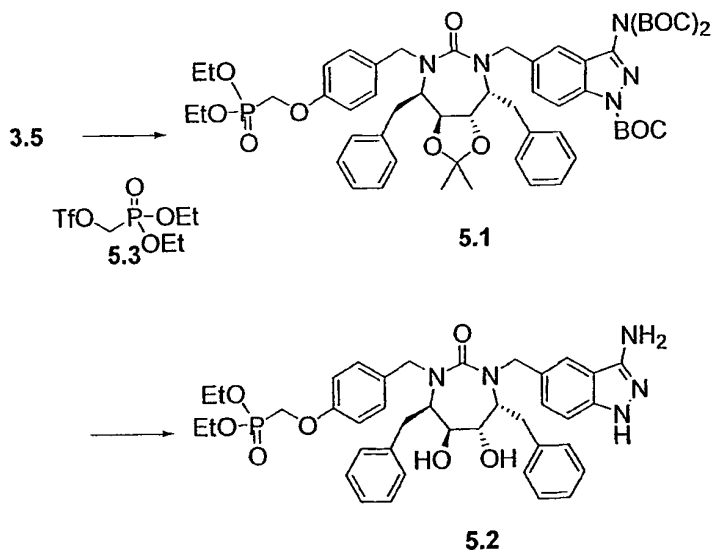
**Phosphonic acid 3.8**: The crude phosphonic acid **3.7** was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and treated with trifluoroacetic acid (0.4 mL). The resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and then purified by preparative HPLC (35 %  $\text{CH}_3\text{CN}$ /65 %  $\text{H}_2\text{O}$ ) to afford the phosphonic acid **3.8** (9.4 mg, 55 %).  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ ):  $\delta$  7.71 (s, 1H), 7.60 (d, 1H), 6.95-7.40 (m, 15H), 4.65 (d, 2H), 4.17 (d, 2H), 3.50-3.70 (m, 3H), 3.42 (d, 1H), 2.03-3.14 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 17.30

## Scheme 4



**Dibenzylphosphonate 4.1:** A solution of **3.6** (30 mg, 25  $\mu$ mol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to afford **4.1** (5 mg, 24%).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  6.96-7.32 (m, 25H), 6.95 (d, 2H), 5.07-5.18 (m, 4H), 4.86 (d, 1H), 4.75 (d, 1H), 4.18 (d, 2H), 3.40-3.62 (m, 4H), 3.25 (d, 1H), 2.80-3.15 (m, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ) 20.5 ppm; MS : 852 (M + H), 874 (M + Na).

## Scheme 5

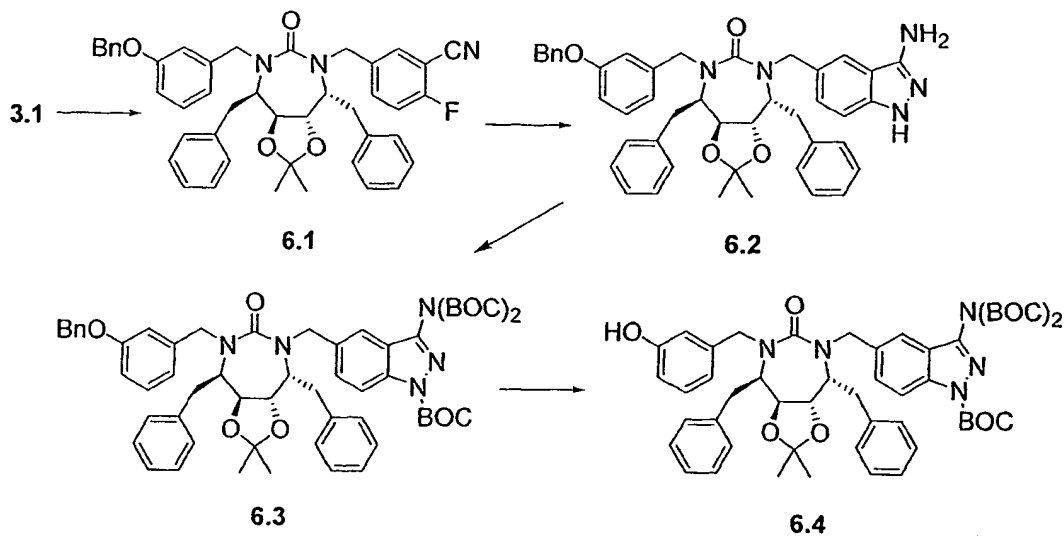


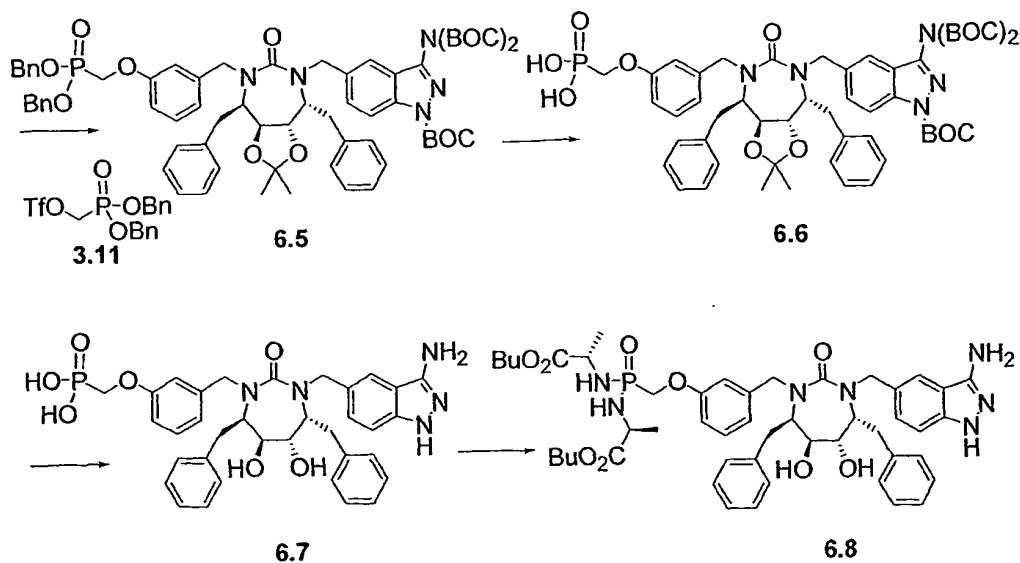
**Diethylphosphonate 5.1:** A solution of phenol **3.5** (48 mg, 52  $\mu$ mol) in THF (5 mL) was treated with triflate **5.3** (50 mg, 165  $\mu$ mol), and cesium carbonate (22 mg, 0.2 mmol). The

resultant mixture was stirred at room temperature for 5 h and then concentrated under reduced pressure. The residue was partitioned between  $\text{CH}_2\text{Cl}_2$  and saturated brine. The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 7% methanol in  $\text{CH}_2\text{Cl}_2$  to afford **5.1** (28 mg, 50 %).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.06 (d, 1H), 7.30-7.43 (m, 7H), 7.02-7.30 (m, 7H), 6.88 (d, 2H), 5.03 (d, 1H), 4.90 (d, 1H), 4.10-4.25 (m, 6H), 3.64-3.80 (m, 4H), 3.20 (d, 1H), 3.05 (d, 1H), 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.20-1.50 (m, 30H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 18.5 ppm; MS : 1068 (M + H), 1090 (M + Na).

**Diethylphosphonate 5.2:** A solution of **5.1** (28 mg, 26  $\mu\text{mol}$ ) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was treated with TFA (0.4 mL) and the resultant mixture was stirred at room temperature for 4 hrs. The mixture was concentrated under reduced pressure and the residue was purified by silica gel to afford **5.2** (11 mg, 55 %).  $^1\text{H}$  NMR ( $\text{CDCl}_3 + 10\% \text{CD}_3\text{OD}$ ):  $\delta$  6.96-7.35 (m, 15H), 6.82 (d, 2H), 4.86 (d, 1H), 4.75 (d, 1H), 4.10-4.23 (m, 6H), 3.40-3.62 (m, 4H), 2.80-3.20 (m), 1.31 (t, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3 + 10\% \text{CD}_3\text{OD}$ ): 19.80 ppm; MS : 728 (M + H).

Scheme 6





**3-Benzyloxybenzyl urea 6.1** : The urea 3.1 (0.87 g, 1.7 mmol) was dissolved in DMF and treated with sodium hydride (60% dispersion, 239 mg, 6.0 mmol) followed by m-benzyloxybenzylbromide 6.9 (0.60 g, 2.15 mmol). The mixture was stirred for 5 h and then diluted with ethyl acetate. The solution was washed with water, brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 25% ethyl acetate in hexanes to afford urea 6.1 (0.9 g, 75%).

**Indazole 6.2**: The urea 6.1 (41 mg, 59  $\mu$ mol) was dissolved in n-butanol (1.5 mL) and treated with hydrazine hydrate (100  $\mu$ L, 100 mmol). The mixture was refluxed for 2 h and then allowed to cool. The mixture was diluted with ethyl acetate, washed with 10% citric acid solution, brine, saturated  $\text{NaHCO}_3$ , and finally brine again. The organic phase was dried over sodium sulfate, filtered and concentrated under reduced pressure to give the crude product 6.2 (35 mg, 83%). (Chem. Biol. 1998, 5, 597-608).

**Boc-indazole 6.3** : The indazole 6.2 (1.04 g, 1.47 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (20 mL) and treated with di-t-butyl dicarbonate (1.28 g, 5.9 mmol), DMAP (0.18 g, 1.9 mmol) and DIPEA (1.02 mL, 9.9 mmol). The mixture was stirred for 3 h and then diluted with ethyl acetate. The solution was washed with 5% citric acid solution,  $\text{NaHCO}_3$ , brine, dried over



magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with 50% ethyl acetate in hexanes to give **6.3** (0.71 g, 49%).

**Phenol 6.4** : Compound **6.3** (20 mg, 0.021 mmol) was dissolved in MeOH (1 mL) and EtOAc (1 mL) and treated with 10% Pd/ C catalyst (5 mg). The mixture was stirred under a hydrogen atmosphere (balloon) until completion. The catalyst was removed by filtration and the filtrate concentrated under reduced pressure to afford compound **6.4** (19 mg, 100%).

**Dibenzyl phosphonate 6.5**: A solution of compound **6.4** (0.34 g, 0.37 mmol) in acetonitrile (5 mL) was treated with Cs<sub>2</sub>CO<sub>3</sub> (0.36 g, 1.1 mmol) and triflate **3.11** (0.18 mL, 0.52 mmol). The reaction mixture was stirred for 1 h. The reaction mixture was filtered and the filtrate was then concentrated under reduced pressure. The residue was re-dissolved in EtOAc, washed with water, saturated NaHCO<sub>3</sub>, and finally brine, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure. The residue was purified by silica gel eluting with hexane: EtOAc (1:1) to afford compound **6.5** (0.32 g, 73%).

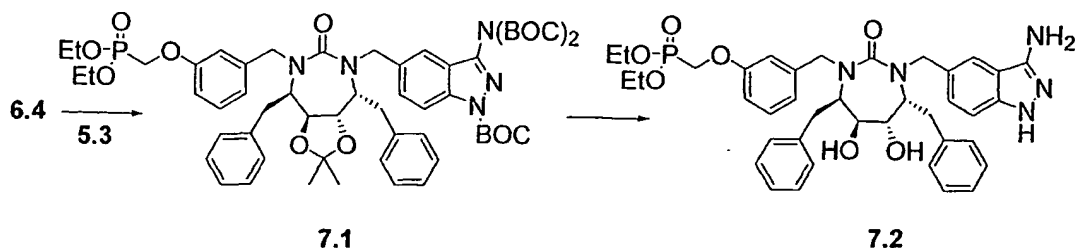
**Phosphonic acid 6.6**: Compound **6.5** (208 mg, 0.174 mmol) was treated in the same manner as benzyl phosphonate **3.6** in the preparation of phosphonate diacid **3.7**, except MeOH was used as the solvent, to afford compound **6.6** (166 mg, 94%).

**Phosphonic acid 6.7**: Compound **6.6** (89 mg, 0.088 mmol) was treated according to the conditions described in Scheme 3 for the conversion of **3.7** into **3.8**. The residue was purified by preparative HPLC eluting with a gradient of 90% methanol in 100 mM TEA bicarbonate buffer and 100% TEA bicarbonate buffer to afford phosphonic acid **6.7** (16 mg, 27%).

**Bisamidate 6.8** : Triphenylphosphine (112 mg, 0.43 mmol) and aldrithiol-2 (95 mg, 0.43 mmol) were mixed in dry pyridine (0.5 mL). In an adjacent flask the diacid **6.7** (48 mg, 0.71 mmol) was suspended in dry pyridine (0.5 mL) and treated with DIPEA (0.075 mL 0.43 mmol) and L-AlaButyl ester hydrochloride (78 mg, 0.43 mmol) and finally the triphenylphosphine, aldrithiol-2 mixture. The reaction mixture was stirred under nitrogen for 24 h then concentrated under reduced pressure. The residue was purified by preparative HPLC eluting with a gradient of 5% to 95% acetonitrile in water. The product obtained was

then further purified by silica gel eluting with  $\text{CH}_2\text{Cl}_2$  : MeOH (9:1) to give compound **6.8** (9 mg, 14%).

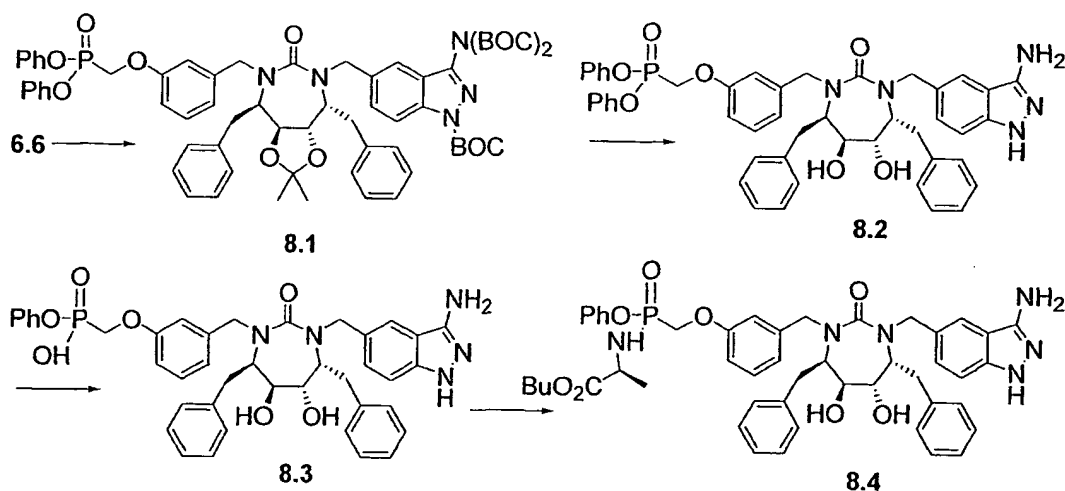
## Scheme 7



**Diethyl phosphonate 7.1 :** Compound 6.4 (164 mg, 0.179 mmol) was treated according to the procedure used to generate compound 6.5 except triflate 5.3 was used in place of triflate 3.11 to afford compound 7.1 (142 mg, 74%).

**Diethylphosphonate 7.2 :** Compound 7.1 (57 mg, 0.053 mmol) was treated according to the conditions used to form 6.7 from 6.6. The residue formed was purified by silica gel eluting with CH<sub>2</sub>Cl<sub>2</sub> : MeOH (9:1) to afford compound 7.2 (13 mg, 33%).

## Scheme 8



**Diphenylphosphonate 8.1:** A solution of 6.6 (0.67g, 0.66 mmol) in pyridine (10 mL) was treated with phenol (0.62 g, 6.6 mmol) and DCC (0.82 mg, 3.9 mmol). The resultant mixture was stirred at room temperature for 5 min and then the solution was heated at 70°C for 3 h. The mixture was allowed to cool to room temperature and then diluted with EtOAc and water

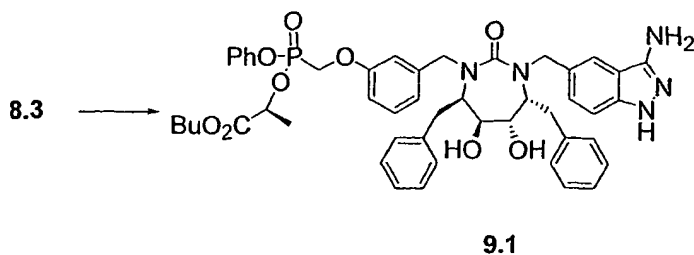
(2 mL). The resultant mixture was stirred at room temperature for 30 min and then concentrated under reduced pressure. The residue was triturated with  $\text{CH}_2\text{Cl}_2$ , and the white solid that formed was removed by filtration. The filtrate was concentrated under reduced pressure and the resultant residue was purified by silica gel eluting with 30% ethyl acetate in hexanes to yield **8.1** (0.5 g, 65 %).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  8.08 (d, 1H), 7.41 (d, 1H), 7.05-7.35 (m, 22H), 6.85 (d, 2H), 6.70 (s, 1H), 5.19 (d, 1H), 5.10 (d, 1H), 4.70 (d, 2H), 3.70-3.90 (m, 4H), 3.20 (d, 1H), 3.11 (d, 1H), 2.80-2.97 (m, 4H), 1.79 (s, 9H), 1.40 (s, 18H), 1.30 (s, 6H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 12.43 ppm

**Diphenylphosphonate 8.2** : A solution of **8.1** (0.5 g, 0.42 mmol) in  $\text{CH}_2\text{Cl}_2$  (4 mL) was treated with TFA (1 mL) and the resultant mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and azeotropeed twice with  $\text{CH}_3\text{CN}$ . The residue was purified by silica gel eluting with 5% methanol in  $\text{CH}_2\text{Cl}_2$  to afford diphenylphosphonate **8.2** (0.25 g, 71 %).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  7.03-7.40 (m, 21H), 6.81-6.90 (m, 3H), 4.96 (d, 1H), 4.90 (d, 1H), 4.60-4.70 (m, 2H), 3.43-3.57 (m, 4H), 3.20 (d, 1H), 2.80-2.97 (m, 5H);  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 12.13 ppm; MS : 824 (M + H).

**Monophenol 8.3** : The monophenol **8.3** (124 mg, 68 %) was prepared from the diphenol **8.2** by treating with 1N NaOH in acetonitrile at  $0^\circ\text{C}$ .

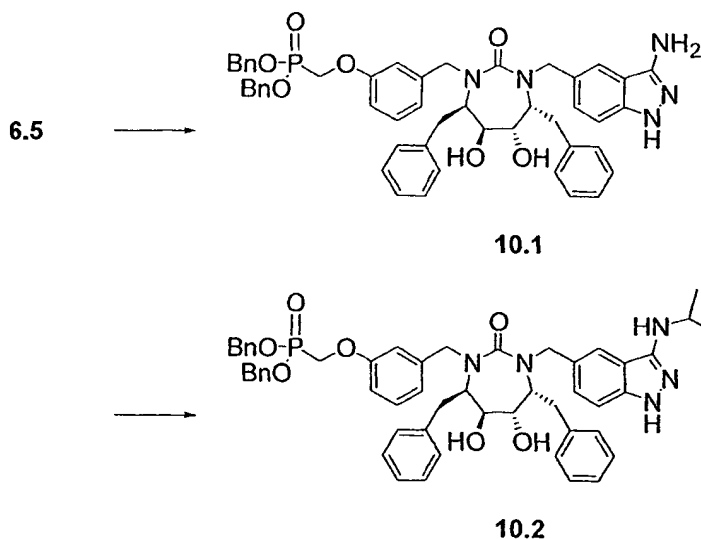
**Monoamidate 8.4** : To a pyridine solution (0.5 mL) of **8.3** (40 mg, 53  $\mu\text{mol}$ ), n-butyl amidate HCl salt (116 mg, 640  $\mu\text{mol}$ ), and DIPEA (83 mg, 640  $\mu\text{mol}$ ) was added a pyridine solution (0.5 mL) of triphenyl phosphine (140 mg, 640  $\mu\text{mol}$ ), and aldrithiol-2 (120 mg, 640  $\mu\text{mol}$ ). The resulting solution was stirred at  $65^\circ\text{C}$  overnight, worked up, and purified by preparative TLC twice to give **8.4** (1.8 mg).  $\delta$  4.96 (d, 1H), 4.90 (d, 1H), 4.30-4.6 (m, 2H), 3.9-4.2 (m, 2H), 3.6-3.70 (m, 4H), 3.2-3.3 (d, 1H), 2.80-3.1 (m, 4H); MS: 875 (M + H) & 897 (M + Na)

## Scheme 9



**Monolactate 9.1:** The monolactate 9.1 is prepared from 8.3 using the conditions described above for the preparation of the monoamidate 8.4 except n-butyl lactate was used in place of n-butyl amidate HCl salt.

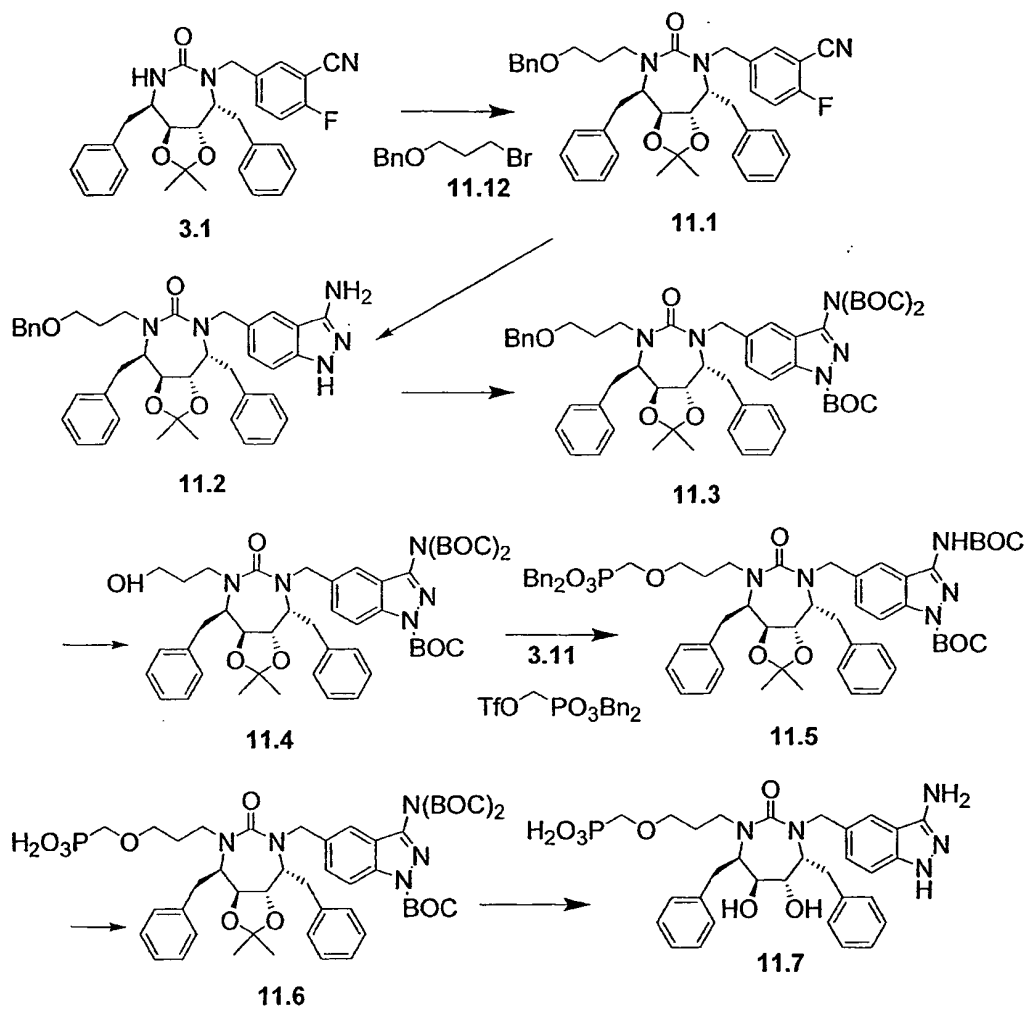
## Scheme 10

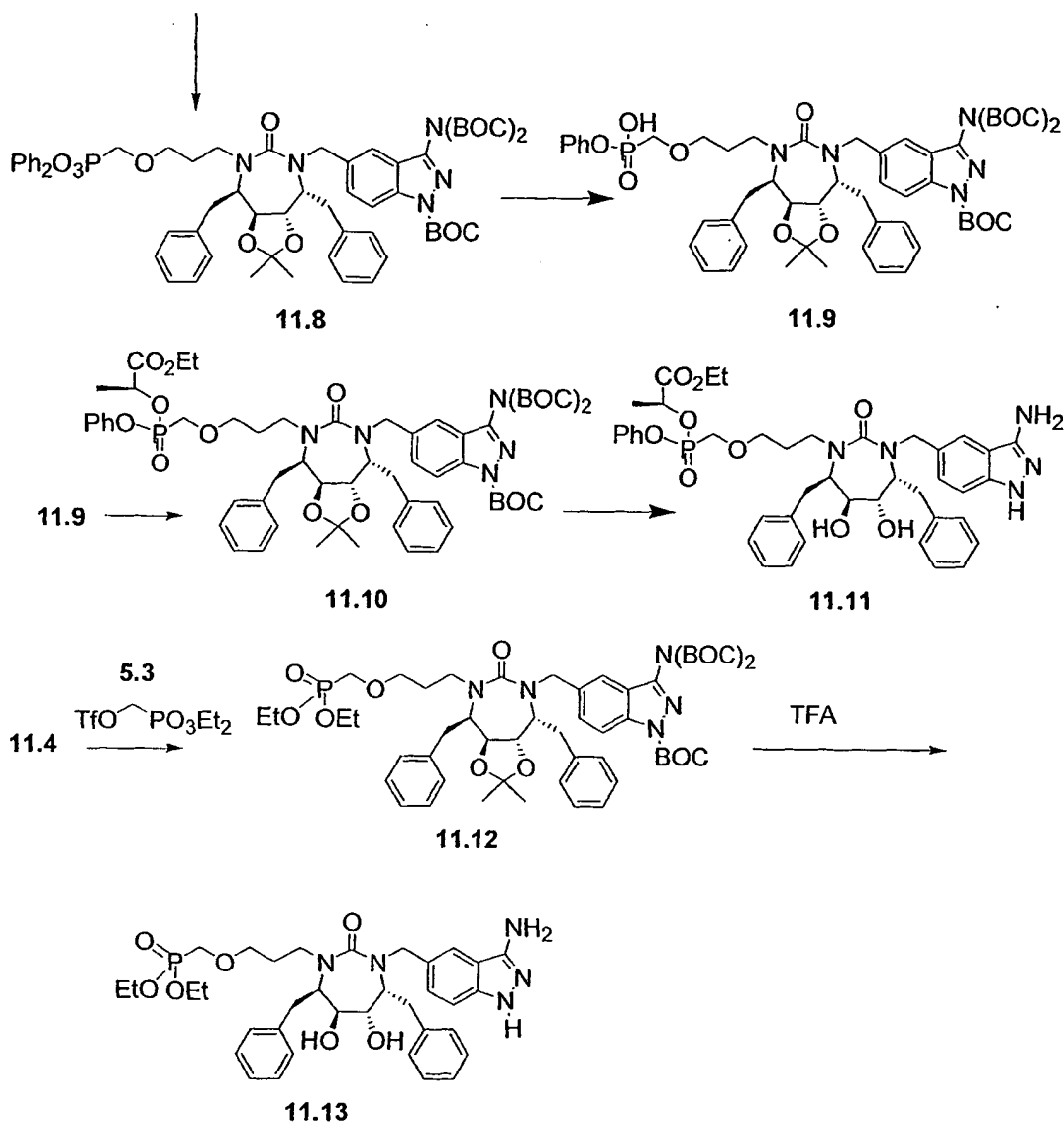


**Dibenzyolphosphonate 10.1:** Compound 6.5 (16 mg, 0.014 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (2 mL) and cooled to  $0^\circ\text{C}$ . TFA (1 mL) was added and the reaction mixture was stirred for 0.5 h. The mixture was then allowed to warm to room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and azeotroped with toluene. The residue was purified by silica gel eluting with  $\text{CH}_2\text{Cl}_2$  : MeOH (9:1) to afford compound 10.1 (4 mg, 32%).

**Isopropylamino indazole 10.2** : Compound **10.1** (30 mg, 0.35 mmol) was treated with acetone according to the method of Henke et al. (J. Med Chem. 40 17 (1997) 2706-2725) to yield **10.2** as a crude residue. The residue was purified by silica gel eluting with CH<sub>2</sub>Cl<sub>2</sub> : MeOH (93:7) to afford compound **10.2** (3.4 mg, 10%).

Scheme 11





**Benzyl ether 11.1:** A DMF solution (5 mL) of **3.1** (0.98 g, 1.96 mmol) was treated with NaH (0.24 g of 60 % oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol), and benzoxypropyl bromide (0.55 g, 2.4 mmol). After the reaction for 3 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **11.1** (0.62 g, 49 %).

**Aminoindazole 11.2:** A n-butanol solution (10 mL) of **11.1** (0.6 g, 0.92 mmol) and hydrazine hydrate (0.93 g, 15.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude **11.2** (~0.6 g).

**Tri-BOC-Aminoindazole 11.3:** A methylene chloride solution (10 mL) of crude **11.2**, DIPEA (0.36 g, 2.8 mmol), (BOC)<sub>2</sub>O (0.73 g, 3.3 mmol), and DMAP (0.34 g, 2.8 mmol) was stirred for 5 h at room temperature, partitioned between methylene chloride and 5 % citric acid solution, dried, purified by silica gel column chromatography to give **11.3** (0.51 g, 58 %, 2 steps).

**3-Hydroxypropyl cyclic urea 11.4:** An ethyl acetate/ethanol solution (30 mL/5 mL) of **11.3** (0.5 g, 0.52 mmol) was hydrogenated at 1 atm in the presence of 10 % Pd/C (0.2 g) for 4 h. The catalyst was removed by filtration. The filtrate was then concentrated under reduced pressure to afford crude **11.4** (0.44 g, 98 %).

**Dibenzyl phosphonate 11.5:** A THF solution (3 mL) of **11.4** (0.5 g, 0.57 mmol) and triflate dibenzyl phosphonate **3.11** (0.37 g, 0.86 mmol) was cooled to -3°C, followed by addition of n-BuLi (0.7 mL of 2.5 M hexane solution, 1.7 mmol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure. The residue was redissolved in methylene chloride (10 mL), and reacted with (BOC)<sub>2</sub>O (0.15 g, 0.7 mmol) in the presence of DMAP (0.18 g, 0.57 mmol), DIPEA (0.18 g, 1.38 mmol) for 2 h at room temperature. The reaction mixture was worked up, and purified by silica gel chromatography to give **11.5** (0.25 g, 43 %).

**Phosphonic diacid 11.7:** An ethyl acetate solution (2 mL) of **11.5A** (11 mg, 10.5 µmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 6 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give crude **11.6**. The crude **11.6** was redissolved in methylene chloride (1 mL) and treated with TFA (0.2 mL) for 4 h at room temperature. The reaction mixture was concentrated under reduced pressure and purified by HPLC to give **11.7** (2 mg, 30%).

NMR (CD<sub>3</sub>OD): δ 7.1-7.3 (m, 11H), 7.0-7.1 (d, 2H), 4.95 (d, 1H), 3.95-4.1 (d, 1H), 2.9-3.3 (m, 4H), 2.3-2.45 (m, 1H), 1.6-1.8 (m, 2H). P NMR (CD<sub>3</sub>OD): 15.5 ppm. MS: 624 (M + 1).

**Diphenyl phosphonate 11.8:** A pyridine solution (1 mL) of **11.6** (0.23 g, 0.23 mmol), phenol (0.27 g, 2.8 mmol), and DCC (0.3 g, 1.4 mmol) was stirred for 5 min. at room



temperature, then reacted at 70°C for 3 h. The reaction mixture was cooled to room temperature, concentrated under reduced pressure, and purified by silica gel column chromatograph to afford **11.8** (0.11 g, 41 %).

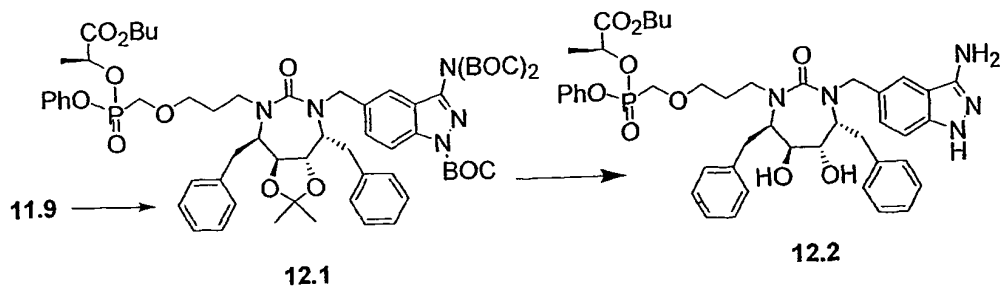
**Monophenyl phosphonate 11.9:** An acetonitrile solution (2 mL) of **11.8** (0.12 g, 0.107 mmol) at 0°C was treated with 1N sodium hydroxide aqueous solution (0.2 mL) for 1.5 h., then acidified with Dowex (50wx8-200, 120 mg). The Dowex was removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was triturated with 10 % EtOAc/90 % hexane twice to afford **11.9** (90 mg, 76 %) as a white solid .

**Mono-ethyl lactate phosphonate 11.10:** A pyridine solution (0.3 mL) of **11.9** (33 mg, 30 µmol), ethyl lactate (41 mg, 340 µmol), and DCC (31 mg, 146 µmol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by silica gel chromatography to give **11.10** (18 mg, 50 %).

**Ethyl lactate phosphonate 11.11:** A methylene chloride solution (0.8 mL) of **11.10** (18 mg, 15.8 µmol) was treated with TFA (0.2 mL) for 4 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **11.11** (6 mg, 50 %). NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): δ 7.0-7.3 (m, 16 H), 6.8-7.0 (m, 2H), 4.9-5.0 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H). 3.5-4.0 (m, 10H), 2.18-2.3. (m, 1H), 1.6-1.7 (m, 1), 1.47 & 1.41 (2d, 3H), 1.22 (t, 3H). P NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): 19.72 & 17.86 ppm.

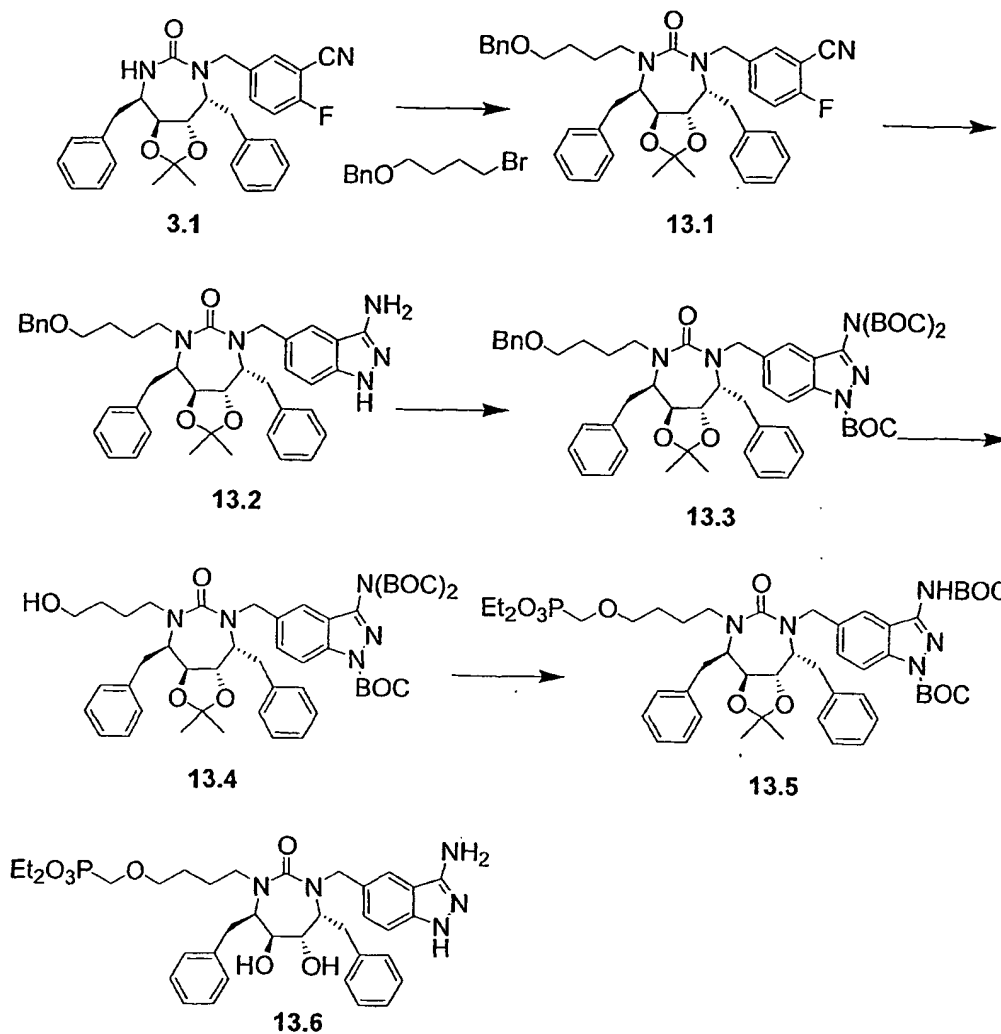
**Diethyl phosphonate 11.13:** Compound **11.13** (6 mg) was prepared as described above in Scheme 5 from **11.4** (30 mg, 34 µmol) and triflate phosphonate **5.3** (52 mg, 172 µmol), followed by TFA treatment. NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): δ 7.1-7.32 (m, 11 H), 6.9-7.0 (d, 2H), 4.75 (d, 1H), 4.1-4.2 (2q, 4H), 3.84-3.9 (m, 1H), 3.4-3.8 (m, 8H), 2.7-3.1 (m, 4H), 2.1-2.5 (m, 1H), 1.5-1.7 (m, 2H), 1.25-1.35 (2t, 6H). P NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): 21.63 ppm. MS: 680 (M + 1).

Scheme 12



**Butyl lactate phosphonate 12.2:** A pyridine solution (0.3 mL) of **11.9** (27 mg, 22  $\mu$ mol), butyl lactate (31 mg, 265  $\mu$ mol), and DCC (28 mg, 132  $\mu$ mol) was stirred at room temperature for 5 min, then reacted at 70°C for 1.5 h. The reaction mixture was concentrated under reduced pressure, partitioned between methylene chloride and saturated NaCl solution, and purified by preparative TLC to give **12.1** (12 mg). A methylene chloride solution (0.8 mL) of **12.1** (12 mg) was treated with TFA (0.2 mL) for 4 h, concentrate. The residue was purified by preparative TLC to give **12.2** (3 mg, 16 %). NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ):  $\delta$  6.8-7.4 (m, 18H), 6.4-6.6 (m), 4.9-5.05 (m, 1H), 4.75 (d, 1H), 4.1-4.2 (m, 2H), 3.5-4.0 (m, 10H), 3.1-3.25 (m, 2H), 2.2-2.35 (m, 1H), 1.8-1.9 (m, 1H), 1.4 & 1.8 (m, 7H), 1.22 (t, 3H). P NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ): 19.69 & 17.86 ppm.

Scheme 13



**Benzyl ether 13.1:** A DMF solution (5 mL) of **3.1** (1 g, 2 mmol) was treated with NaH (0.24 g of 60% oil dispersion, 6 mmol) for 30 min, followed by the addition of sodium iodide (0.3 g, 2 mmol), and benzyloxybutyl bromide (0.58 g, 2.4 mmol). After the reaction for 5 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **13.1** (0.58 g, 44 %).

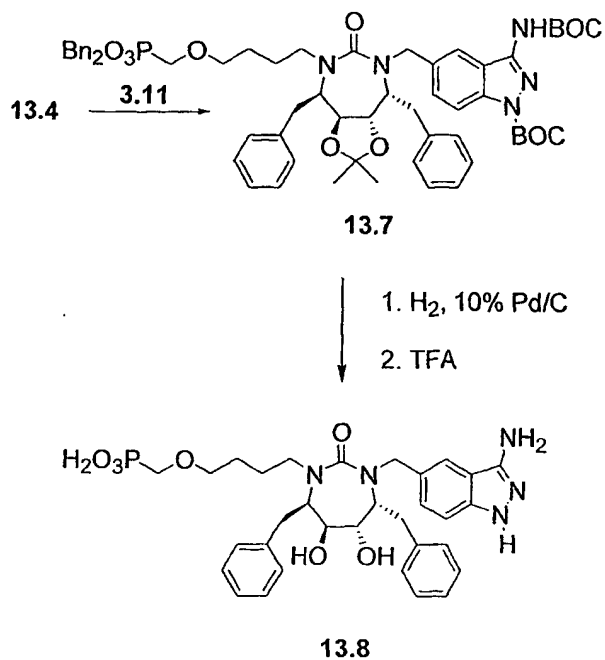
**Aminoindazole 13.2:** A n-butanol solution (10 mL) of **11.1** (0.58 g, 0.87 mmol) and hydrazine hydrate (0.88 g, 17.5 mmol) was heated at reflux for 4 h. The reaction mixture was concentrated under reduced pressure to give crude **13.2** (0.56 g).

**Tri-BOC-aminoindazole 13.3:** A methylene chloride solution (10 mL) of **13.2** (0.55 g, 0.82 mmol), DIPEA (0.42 g, 3.2 mmol), (BOC)<sub>2</sub>O (0.71 g, 3.2 mmol), and DMAP (0.3 g, 2.4 mmol) was stirred for 4 h at room temperature, partitioned between methylene chloride and 5% citric acid solution, dried, purified by silica gel chromatography to give **13.3** (0.56 g, 71 %, 2 steps).

**3-Hydroxybutyl cyclic urea 13.4:** An ethyl acetate/methanol solution (30 mL/5 mL) of **11.3** (0.55 g, 0.56 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (0.2 g) for 3 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford crude **13.4** (0.5 g, 98 %).

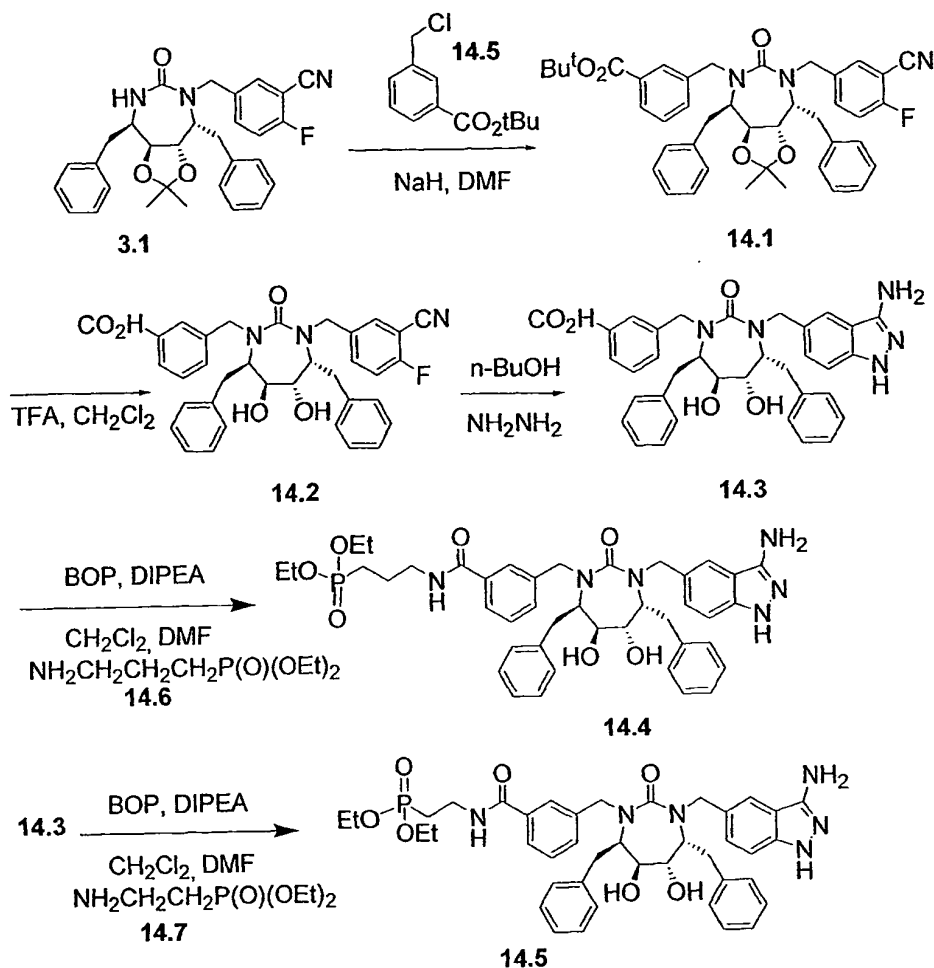
**Diethyl phosphonate 13.6:** A THF solution (1 mL) of **13.4** (5 mg, 56 µmol) and triflate diethyl phosphonate **5.3** (30 mg, 100 µmol) was cooled to -3°C, followed by addition of n-BuLi (80 µl of 2.5 M hexane solution, 200 µmol). After 2 h reaction, the reaction mixture was partitioned between methylene chloride and saturated NaCl solution, concentrated under reduced pressure to give crude **13.5**. The residue was dissolved in methylene chloride (0.8 mL) and treated with TFA (0.2 mL) for 4 h. concentrated under reduced pressure, and purified by HPLC to give **13.6** (8 mg, 21%). NMR (CDCl<sub>3</sub>): δ 7.1-7.4 (m, 1H), 7.0-7.1 (m, 2H) 4.81 (d, 1H), 4.1-4.25 (m, 4H). 3.85-3.95 (m, 1H), 3.4-3.8 (m, 7H), 3.3-3.4 (m, 1H), 2.8 - 3.25 (m, 5H), 2.0-2.15 (m, 1H), 1.3-1.85 (m, 10H). P NMR (CDCl<sub>3</sub>): 21.45 ppm.

## Scheme 13a



**Phosphonic diacid 13.8:** Compound 13.8 (4.5 mg) was prepared from 13.4 as described above for the preparation of 11.7 from 11.4 (Scheme 11). NMR (CD<sub>3</sub>OD):  $\delta$  7.41 (s, 1H), 7.1-7.4 (m, 10H), 6.9-7.0 (m, 2H), 4.75 (d, 1H), 3.8-4.0 (m, 1H), 3.4-3.8 (m, 8H), 2.8-3.25 (m, 5H), 2.1-2.25 (m, 1H), 1.6-1.85 (m, 4H). MS: 638 (M + 1).

Scheme 14



**t-Butyl ester 14.1:** A DMF solution (3 mL) of **3.1** (0.5 g, 1 mmol) was treated with NaH (80 mg of 60% oil dispersion, 2 mmol) for 10 min, followed by the addition of **14.5** (0.25 g, 1.1 mmol). After the reaction for 1 h at room temperature, the reaction mixture was partitioned between methylene chloride and saturated NaCl, dried, and purified to give **14.1** (0.4 g, 59%).

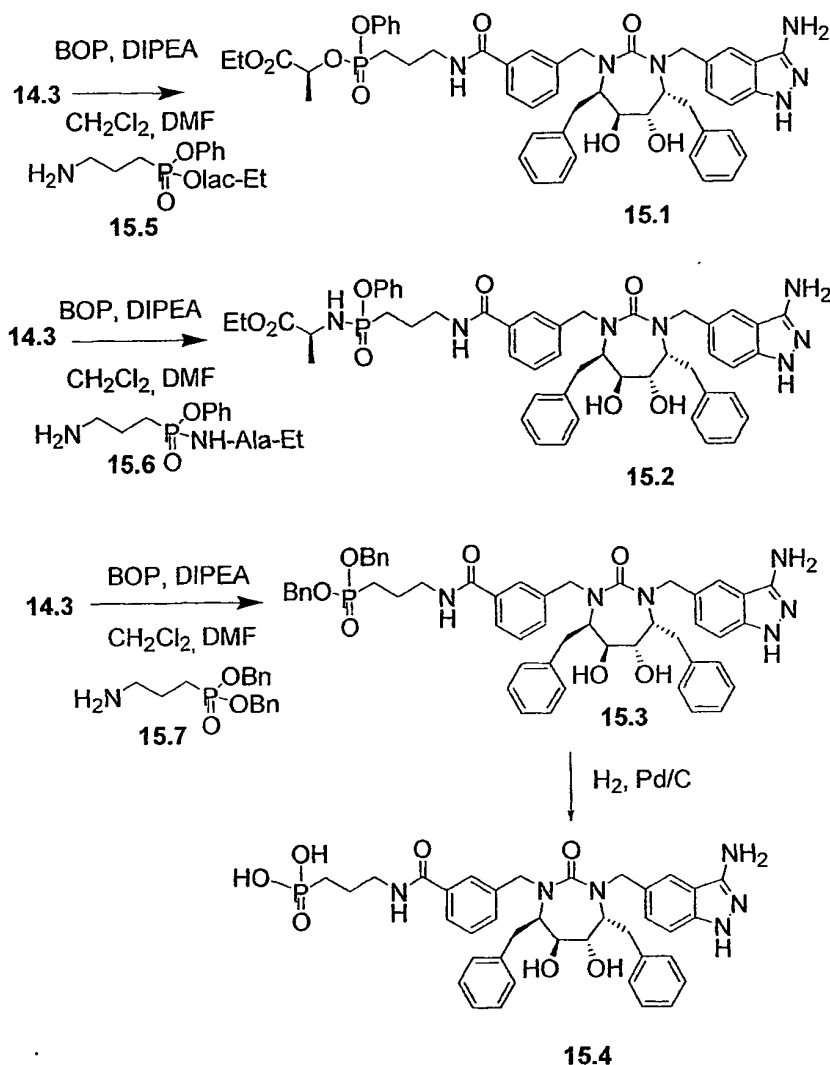
**Aminoindazole derivative 14.3:** A methylene chloride solution (5 mL) of **14.1** (0.4 g, 0.58 mmol) was treated with TFA (1 mL) at room temperature for 1.5 h, and then concentrated under reduced pressure to give crude **14.2**. The crude **14.2** was dissolved in n-BuOH (5 mL) and reacted with hydrazine hydrate (0.58 g, 11.6 mmol) at reflux for 5 h. The reaction

mixture was concentrated under reduced pressure and purified by silica gel chromatography to give the desired product **14.3** (0.37 g, quantitative yield).

**Diethylphosphonate ester 14.4:** A methylene chloride solution (3 mL) of **14.3** (23 mg, 38  $\mu\text{mol}$ ) was reacted with aminopropyl-diethylphosphonate **14.6** (58 mg, 190  $\mu\text{mol}$ ), DIPEA (50 mg, 380  $\mu\text{mol}$ ), and ByBOP (21 mg, 48  $\mu\text{mol}$ ) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **14.4** (9 mg, 34 %). NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.87 (t, 1H), 7.61 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 10 H), 6.93-7.0 (m, 4H), 4.79 (d, 2H), 3.99-4.04 (m, 4H), 3.38-3.65 (m, 6H), 2.60-3.2 (m, 6 H), 1.70-1.87 (m, 4H), 1.25 (t, 6H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 32.7 ppm.

**Diethylphosphonate ester 14.5:** A methylene chloride solution (2 mL) of **14.3** (13 mg, 21  $\mu\text{mol}$ ) was reacted with aminoethyl-diethylphosphonate oxalate **14.7** (23mg, 85  $\mu\text{mol}$ ), DIPEA (22 mg, 170  $\mu\text{mol}$ ), and ByBOP (12 mg, 25  $\mu\text{mol}$ ) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **14.5** (5mg, 30%). Ms: 783 ( $M + 1$ ). NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.88 (b, 1H), 7.58 (b, 1H), 7.49 (s, 1H), 7.14-7.2 (m, 10 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.90-4.04 (m, 4H), 2.50-3.3 (m, 6 H), 1.97-2.08 (m, 2H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 30.12 ppm.

## Scheme 15



**Monophenol-ethyl lactate phosphonate prodrug 15.1:** A methylene chloride/DMF solution (2 mL/0.5 mL) of 14.3 (30 mg, 49  $\mu$ mol) was reacted with aminopropyl-phenol-ethyl lactate phosphonate 15.5 (100 mg, 233  $\mu$ mol), DIPEA (64 mg, 495  $\mu$ mol), and BOP reagent (45 mg, 100  $\mu$ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by silica gel chromatography to give 15.1 (28 mg, 64 %). NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>O):  $\delta$  7.83 (b, 1H), 7.59 (b, 1H), 7.51 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75-4.87 (d +



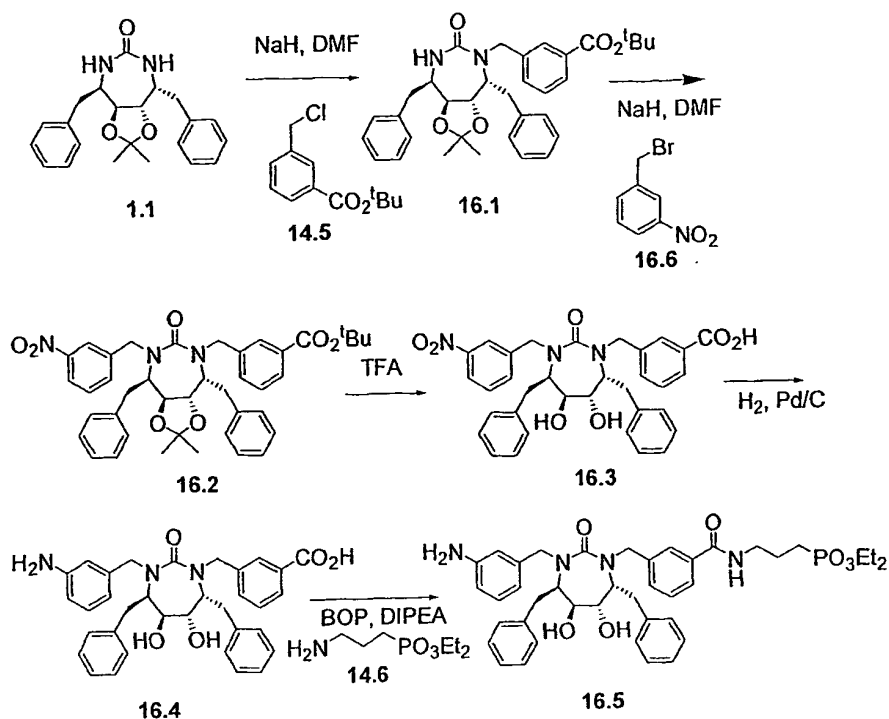
q, 3H), 4.10 (q, 2H), 3.3-3.61 (m, 6H), 2.60-3.2 (m, 6H), 1.92-2.12 (m, 4H), 1.30 (d, 3H), 1.18 (t, 3H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 30.71 ppm. MS: 903 ( $M + 1$ ).

**Phenol-ethyl alanine phosphonate prodrug 15.2:** A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49  $\mu\text{mol}$ ) was reacted with aminopropyl-phenol-ethyl alanine phosphonate **15.6** (80 mg TFA salt, 186  $\mu\text{mol}$ ), DIPEA (64 mg, 500  $\mu\text{mol}$ ), and BOP reagent (45 mg, 100  $\mu\text{mol}$ ) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.2** (12 mg, 27 %). NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.91 (b, 1H), 7.61 (b, 1H), 7.52 (s, 1H), 7.14-7.2 (m, 11 H), 6.90-7.0 (m, 4H), 4.75 (d, 2H), 3.82-4.1 (2q, 3H), 3.4-3.65 (m, 6H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H), 1.3 (d, 3H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 32.98 & 33.38 ppm. MS: 902 ( $M + 1$ ).

**Dibenzyl phosphonate 15.3:** A methylene chloride/DMF solution (2 mL/0.5 mL) of **14.3** (30 mg, 49  $\mu\text{mol}$ ) was reacted with aminopropyl dibenzyl phosphonate **15.7** (86 mg TFA salt, 200  $\mu\text{mol}$ ), DIPEA (64 mg, 500  $\mu\text{mol}$ ), and BOP reagent (45 mg, 100  $\mu\text{mol}$ ) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was triturated with methylene chloride/hexane. The solid was purified by preparative TLC to give **15.3** (20 mg, 44%). NMR ( $\text{CDCl}_3 + \sim 5\% \text{CD}_3\text{O}$ ):  $\delta$  7.50-7.58 (m, 2H), 7.14-7.3 (m, 21 H), 6.90-7.0 (m, 4H), 4.7-5.1 (m, 6H), 3.6-3.8 (m, 4H), 3.3-3.55 (m, 2H), 2.60-3.15 (m, 6H), 1.8-2.0 (m, 4H). P NMR ( $\text{CDCl}_3 + \sim 5\% \text{CD}_3\text{OD}$ ): 33.7 ppm. MS: 907 ( $M + 1$ ).

**Phosphonic diacid 15.4:** An ethanol solution (5 mL) of **15.3** (17 mg, 18.7  $\mu\text{mol}$ ) was hydrogenated at 1 atm in the presence of 10 % Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **15.4** (12 mg, 85%). NMR ( $\text{CD}_3\text{O} + 20\% \text{CDCl}_3$ ):  $\delta$  7.88 (b, 1H), 7.59 (b, 1H), 7.6 (s, 1H), 7.1-7.25 (m, 10 H), 6.90-7.1 (m, 4H), 4.8 (d, 2H + water peak), 3.6-3.8 (m, 4H), 3.4-3.5 (m, 2H), 1.85-2.0 (m, 4H).

Scheme 16



**Monobenzyl derivative 16.1:** A DMF solution (4 mL) of 1.1 (0.8 g, 2.2 mmol) was treated with NaH (0.18 g of 60% oil dispersion, 4.4 mmol) for 10 min at room temperature followed by the addition of 14.5 (0.5 g, 2.2 mmol). The resulting solution was reacted at room temperature for 2 h, worked up, and then purified to afford 16.1 (0.48 g, 40%).

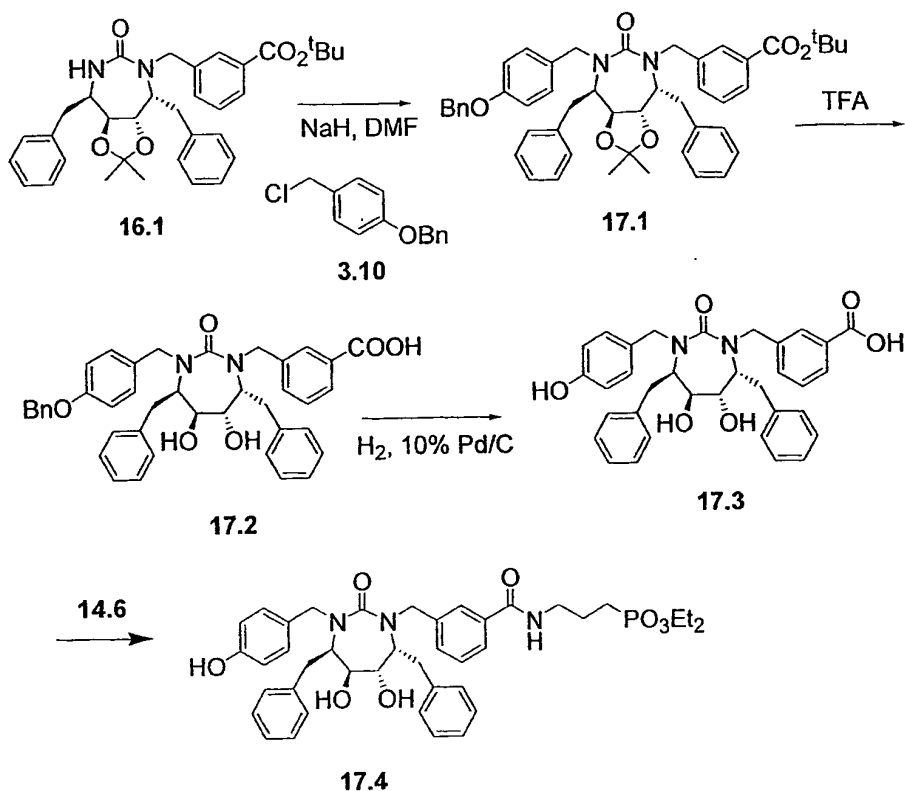
**3-Nitrobenzyl cyclic urea derivative 16.2:** A DMF solution (0.5 mL) of 16.1 (65 mg, 117  $\mu$ mol) was treated with NaH (15 mg of 60% oil dispersion, 375  $\mu$ mol) for 10 min at room temperature, followed by the addition of 3-nitrobenzyl bromide (33 mg, 152  $\mu$ mol). The resulting solution was reacted at room temperature for 1 h, worked up, and purified by preparative TLC to afford 16.2 (66 mg, 82%).

**Diol 16.3:** A methylene chloride solution (2 mL) of 16.2 (46 mg, 61  $\mu$ mol) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to afford 16.3. This material was used without further purification.

**3-Aminobenzyl cyclic urea 16.4:** An ethyl acetate/ethanol (5 mL/1 mL) solution of **16.3** (crude) was hydrogenated at 1 atm in the presence of 10% Pd/C for 2 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to afford **16.4** (26 mg, 70%, 2 steps).

**Diethyl phosphonate 16.5:** A methylene chloride/DMF solution (2 mL/0.5 mL) of **16.4** (24 mg, 42  $\mu$ mol) was reacted with aminopropyl-diethylphosphonate ester TFA salt **14.6** (39 mg, 127  $\mu$ mol), DIPEA (27 mg, 210  $\mu$ mol), and BOP reagent (28 mg, 63  $\mu$ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give **16.5** (20.7 mg, 63 %). NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.62 (b, 1H), 7.51 (s, 1H), 7.0-7.35 (m, 12 H), 6.95 (d, 2H), 6.85 (d, 2H), 4.6-4.71 (2d, 2H), 3.95-4.1 (m, 4H), 3.3-3.55 (m, 3H), 2.60-2.8 (m, 2H), 2.95-3.15 (m, 4 H), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 32.65 ppm.

Scheme 17

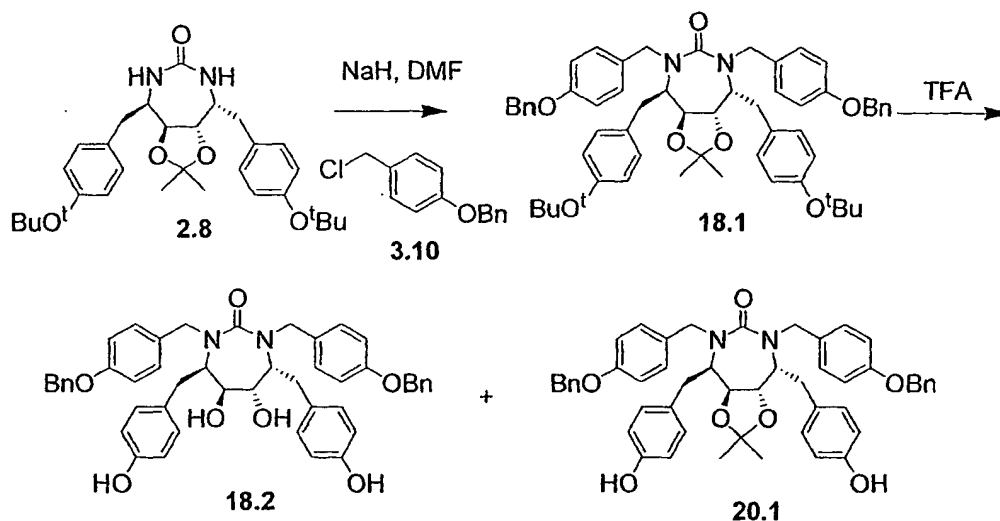


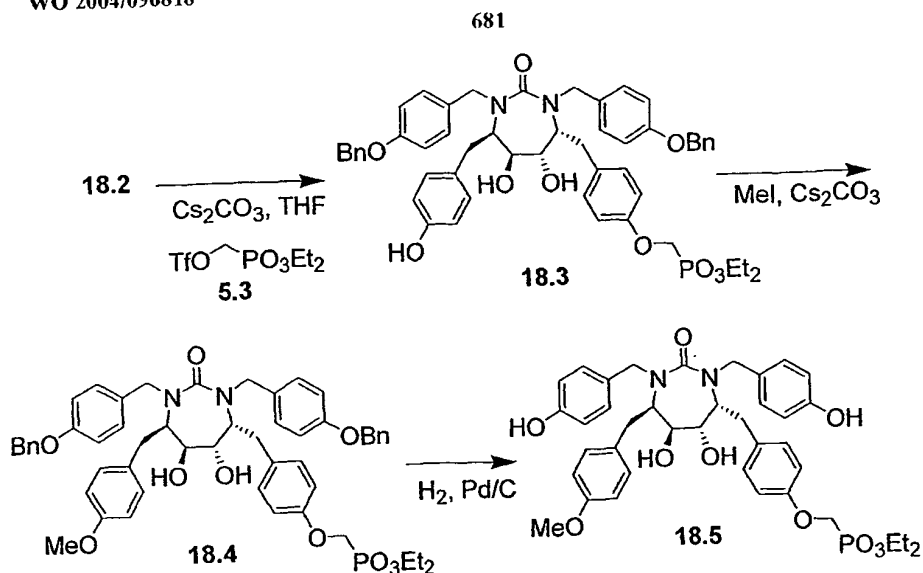
**p-Benzoybenzyl cyclic urea derivative 17.1:** A DMF solution (0.5 mL) of 16.1 (65 mg, 117  $\mu\text{mol}$ ) was treated with NaH (15 mg of 60% oil dispersion, 375  $\mu\text{mol}$ ) for 10 min at room temperature, followed by the addition of 4-benzyloxy benzyl chloride 3.10 (35 mg,  $\mu\text{mol}$ ). The resulting solution was stirred for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure, purified by preparative TLC to generate 17.1 (62 mg, 70%).

**Diethyl phosphonate 17.3:** A methylene chloride solution (2 mL) of 17.1 (46 mg, 61  $\mu\text{mol}$ ) was treated with TFA (0.4 mL) for 2 h at room temperature, and then concentrated under reduced pressure to give crude 17.2. An ethyl acetate/ethanol solution (3 mL/2 mL) of the crude 17.2 was then hydrogenated at 1 atm in the presence of 10% Pd/C (10 mg) for 5 h at room temperature. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure to afford 17.3 (crude).

**Diethyl phosphonate cyclic urea 17.4:** A methylene chloride/DMF solution (2 mL/0.5 mL) of 17.3 (25 mg, 42  $\mu$ mol) was reacted with aminopropyl-diethylphosphonate ester TFA salt 14.6 (40 mg, 127  $\mu$ mol), DIPEA (27 mg, 210  $\mu$ mol), and BOP reagent (28 mg, 63  $\mu$ mol) at room temperature for 2 h, and then concentrated under reduced pressure. The residue was purified by preparative TLC to give 17.4 (14.6 mg, 44 %). NMR ( $\text{CDCl}_3$  +  $\sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.82 (t), 7.62 (d, 1H), 7.51 (s, 1H), 7.05-7.35 (m, 10 H), 6.8-6.95 (2d, 4H), 6.85 (d, 2H), 4.8 (d, 1H), 4.65 (d, 1H), 3.95-4.1 (m, 4H), 3.4-3.75 (m, 6H), 2.60-3.2 (m), 1.85-2.0 (m, 4H), 1.25 (t, 6H). P NMR ( $\text{CDCl}_3$  +  $\sim 10\% \text{CD}_3\text{OD}$ ): 32.72 ppm.

Scheme 18





**Dibenzyl derivative 18.1:** A DMF solution (3 mL) of compound 2.8 (0.4 g, 0.78 mmol) was reacted with 60%NaH (0.13 g, 1.96 mmol), 4-benzyloxy benzylchloride 3.10 (0.46 g, 1.96 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 4 h. The reaction mixture was partitioned between methylene chloride and saturated NaHCO<sub>3</sub> solution. The organic phase was isolated, dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product 18.1 (0.57 g, 81%).

**Diol derivative 18.2 and diphenol derivative 20.1:** A methylene chloride solution (4 mL) of 18.1 (0.57 g, 0.63 mmol) was treated with TFA (1 mL) at room temperature for 20 min, concentrated under reduced pressure, and purified by silica gel chromatography to give diol derivative 18.2 (133 mg, 28 %) and diphenol derivative 20.1 (288 mg, 57.6%).

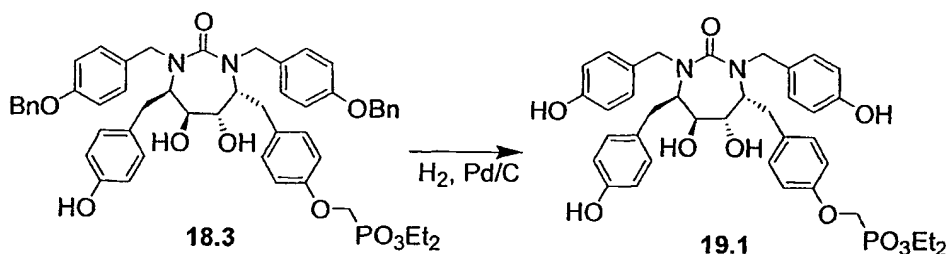
**Monophosphonate derivative 18.3:** A THF solution (10 mL) of 18.2 (130 mg, 0.17 mmol) was stirred with cesium carbonate (70 mg, 0.21 mmol) and diethylphosphonate triflate 5.3 (52 mg, 0.17 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give 18.3 (64 mg, 41 %), and recovered 18.2 (25 mg, 19%).

**Methoxy derivative 18.4:** A THF solution (2 mL) of 18.3 (28 mg, 25 µmol) was treated with cesium carbonate (25 mg, 76 µmol) and iodomethane (10 eq. Excess) at room

temperature for 5 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated  $\text{NaHCO}_3$ . The organic phase was separated, concentrated under reduced pressure and the residue purified by preparative TLC to afford **18.4** (22 mg, 78%).

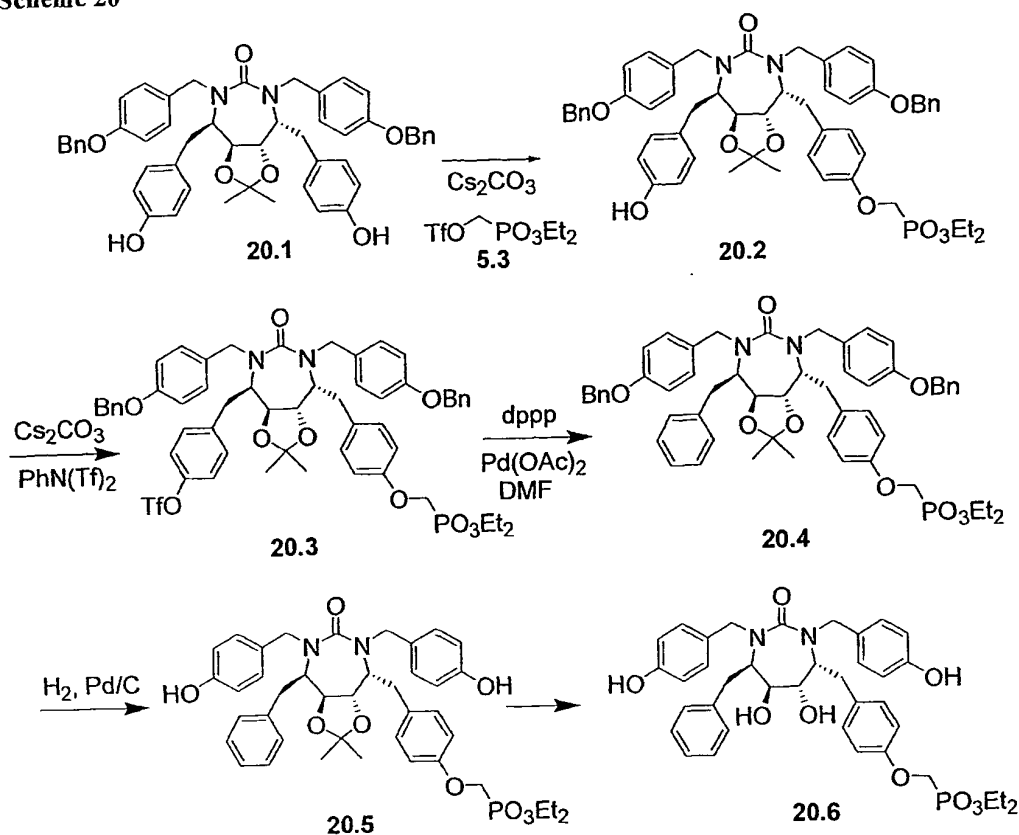
**Diethylphosphonate 18.5** : An ethyl acetate/ethanol (2 mL/2 mL) solution of **18.4** (22 mg, 24  $\mu\text{mol}$ ) was hydrogenated at 1 atm in the presence of 10% Pd/C for 3 h. The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure to give the desired product **18.5** (18 mg, quantitative). NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{O}$ ):  $\delta$  6.7-7.0 (m, 12 H), 6.62-6.69 (m, 4H), 4.65 (d, 1H), 4.50 (d, 1H), 4.18-4.3 (m, 6H), 3.75 (s, 3H), 3.3-3.4 (m, 4H), 2.8-3.0 (m, 6H), 1.30 (t, 6H). P NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ): 20.16 ppm.

Scheme 19



**Diethyl phosphonate 19.1**: An ethyl acetate/ethanol (2 mL/1 mL) solution of **18.3** (14 mg, 15.5  $\mu\text{mol}$ ) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was then removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **19.1** (10 mg, 90%). NMR ( $\text{CDCl}_3$  + ~15 %  $\text{CD}_3\text{O}$ ):  $\delta$  6.6-7.0 (m, 16 H), 4.5-4.65 (2d, 2H), 4.1-4.3 (m, 6H), 2.7-3.0 (m, 6H), 1.29 (t, 6H). P NMR ( $\text{CDCl}_3$  + ~15 %  $\text{CD}_3\text{OD}$ ): 20.12 ppm.

Scheme 20



**Monophosphonate 20.2:** A THF solution (8 mL) of 20.1 (280 mg, 0.36 mmol) was stirred with cesium carbonate (140 mg, 0.43 mmol) and diethylphosphonate triflate 5.3 (110 mg, 0.36 mmol) at room temperature for 4 h.. The reaction mixture was concentrated under reduced pressure and purified to give 20.2 (130mg, 39%), and recovered 20.1 (76 mg, 27%).

**Triflate derivative 20.3:** A THF solution (6 mL) of 20.2 (130 mg, 0.13 mmol) was stirred with cesium carbonate (67 mg, 0.21 mmol) and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give 20.3 (125 mg, 84%).

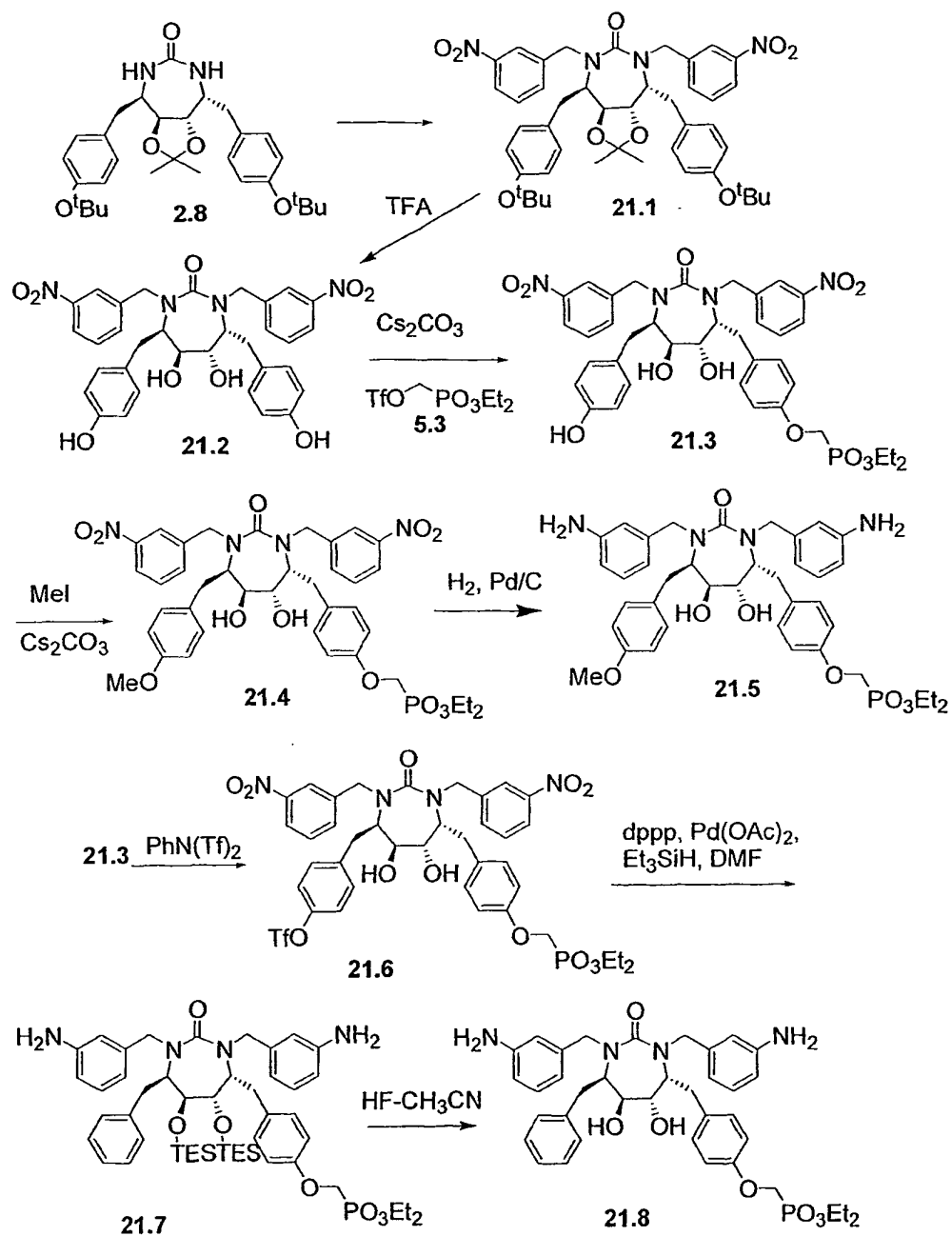
**Benzyl ether 20.4:** To a DMF solution (2 mL) of  $\text{Pd}(\text{OAc})_2$  (60 mg, 267  $\mu\text{mol}$ ), and dppp (105 mg, 254  $\mu\text{mol}$ ) was added 20.3 (120 mg, 111  $\mu\text{mol}$ ) under nitrogen, followed by the addition of triethylsilane (0.3 mL). The resulting solution was stirred at room temperature for



4 h, then concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford **20.4** (94 mg, 92%).

**Diethyl phosphonate 20.6:** An ethyl acetate/ethanol (2 mL/2 mL) solution of **20.4** (28 mg, 30  $\mu$ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C (5 mg) for 3 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **20.5**. The crude product **20.5** was redissolved in methylene chloride (2 mL) and treated with TFA (0.4 mL) and a drop of water. After 1 h stirring at room temperature, the reaction mixture was concentrated under reduced pressure, and purified by preparative TLC plate to give **20.6** (18 mg, 85 %, 2 steps).  $\delta$  6.6-7.3 (m, 17 H), 4.65 (d, 1H), 4.58 (d, 1H), 4.18-4.3 (m, 6H), 3.3-3.5 (m, 4H), 2.8-3.1 (m), 1.34 (t, 6H). P NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ): 20.16 ppm. MS: 705 (M + 1).

Scheme 21



**Bis-(3-nitrobenzyl) derivative 21.1:** A DMF solution (2 mL) of compound 2.8 (0.3 g, 0.59 mmol) was reacted with 60%NaH (0.07 g, 1.76 mmol), 3-nitrobenzyl bromide (0.38 g, 1.76 mmol) and sodium iodide (60 mg, 0.39 mmol) at room temperature for 3 h. The reaction

mixture was partitioned between methylene chloride and saturated  $\text{NaHCO}_3$  solution. The organic phase was isolated, dried over  $\text{Na}_2\text{SO}_4$ , concentrated under reduced pressure, and purified by silica gel chromatography to give the desired product **21.1** (0.37 g, 82%).

**Diphenol derivative 21.2:** A methylene chloride solution (4 mL) of **21.1** (0.37 g, 0.47 mmol) was treated with TFA (1 mL) at room temperature for 3 h, and then concentrated under reduced pressure, and azeotroped with  $\text{CH}_3\text{CN}$  twice to give diphenol derivative **21.2** (0.3 g, quantitative).

**Monophosphonate derivative 21.3:** A THF solution (8 mL) of **18.2** (0.28g, 0.44 mmol) was stirred with cesium carbonate (0.17 g, 0.53 mmol) and diethylphosphonate triflate **5.3** (0.14 g, 0.44 mmol) at room temperature for 4 h. The reaction mixture was concentrated under reduced pressure and purified to give **21.3** (120 mg, 35%), and recovered **21.2** (150 mg, 53%).

**Methoxy derivative 21.4:** A THF solution (2 mL) of **21.3** (9 mg, 11  $\mu\text{mol}$ ) was treated with cesium carbonate (15 mg, 46  $\mu\text{mol}$ ) and iodomethane (10 eq. Excess) at room temperature for 6 h. The reaction mixture was concentrated under reduced pressure and partitioned between methylene chloride and saturated  $\text{NaHCO}_3$ . The organic phase was separated, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by preparative TLC to afford **21.4** (9 mg)

**Diethylphosphonate 21.5:** A ethyl acetate/ethanol (2 mL/0.5 mL) solution of **21.4** (9 mg, 11  $\mu\text{mol}$ ) was hydrogenated at 1 atm in the presence of 10% Pd/C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under reduced pressure to give the desired product **21.5** (4.3 mg, 49%, 2 steps). NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{O}$ ):  $\delta$  7.0-7.10 (m, 6 H), 6.8-6.95 (m, 4H), 6.5-6.6 (m, 4H), 6.4-6.45 (m, 2H), 4.72 (d, 2H), 4.18-4.3 (m, 6H), 3.72 (s, 3H), 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ): 19.93 ppm.

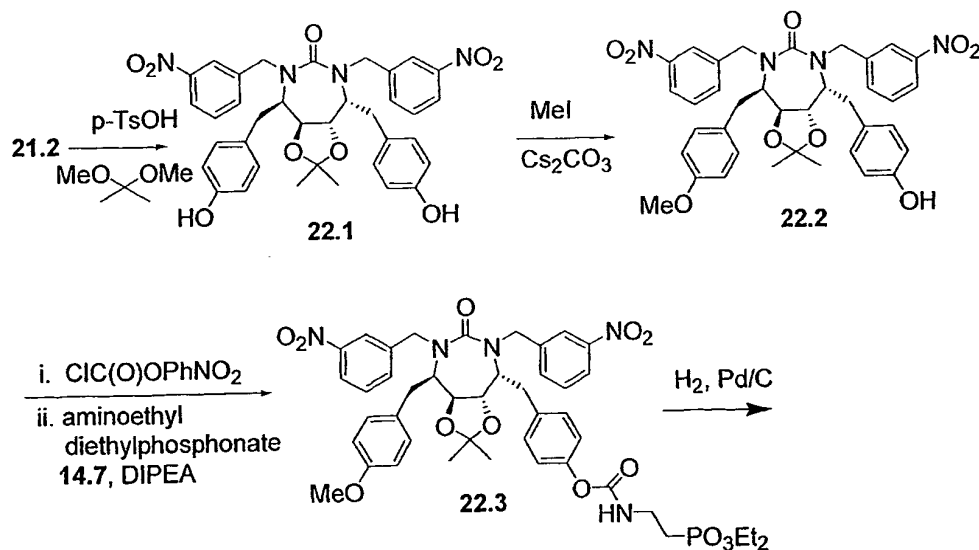
**Triflate 21.6:** A THF solution (6 mL) of **21.3** (0.1g, 0.14 mmol), cesium carbonate (0.07 g, 0.21 mmol), and N-phenyltrifluoromethane-sulfonimide (60mg, 0.17 mmol) was stirred at

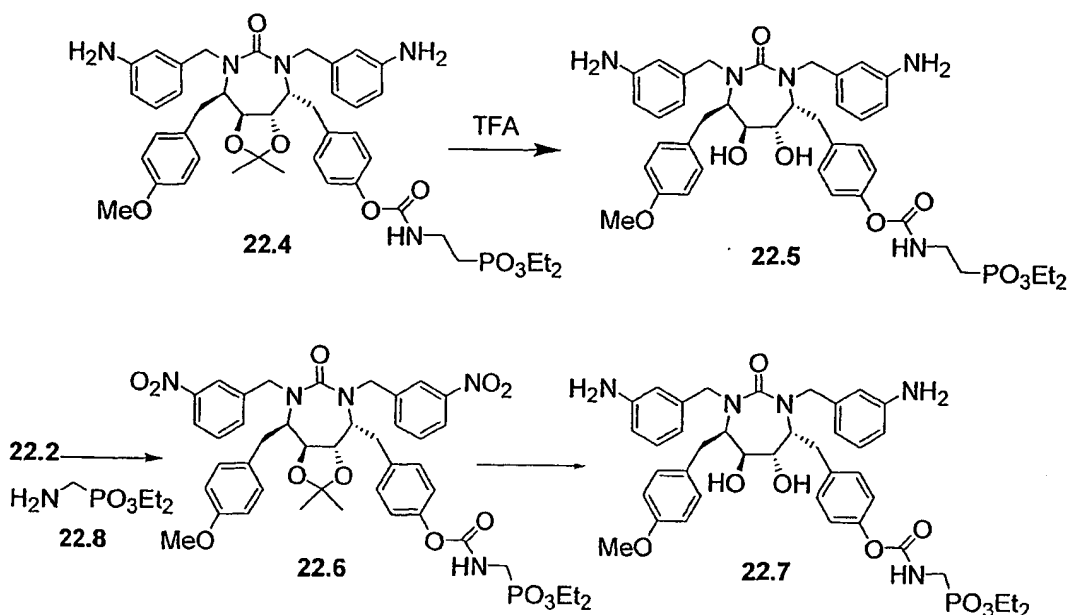
room temperature for 4 h, and then concentrated under reduced pressure, and worked up. The residue was purified by silica gel chromatography to give **21.6** (116 mg, 90%).

**Diamine 21.7:** A DMF solution (2 mL) of **21.6** (116 mg, 127  $\mu\text{mol}$ ), dppp (60 mg, 145  $\mu\text{mol}$ ), and  $\text{Pd}(\text{OAc})_2$  (30 mg, 134  $\mu\text{mol}$ ) was stirred under nitrogen, followed by addition of triethylsilane (0.3 mL), and reacted for 4 h at room temperature. The reaction mixture was worked up and purified to give **21.7** (50 mg).

**Diethyl phosphonate 21.8:** An acetonitrile solution (1 mL) of crude **21.7** (50 mg) was treated with 48% HF (0.1 mL) for 4 h. The reaction mixture was concentrated under reduced pressure, and purified to give **21.8** (10 mg, 11% (2 steps). NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{O}$ ):  $\delta$  7.05-7.30 (m, 9 H), 6.8-6.95 (d, 2H), 6.4-6.6 (m, 6H), 4.72 (d, 2H), 4.18-4.3 (m, 6H), 3.4-3.5 (m, 4H), 2.8-3.0 (m, 6H), 1.34 (t, 6H). P NMR ( $\text{CDCl}_3 + \sim 10\% \text{CD}_3\text{OD}$ ): 19.83 ppm.

Scheme 22





**Acetonide 22.1:** An acetone/2,2-dimethoxypropane solution (15 mL/5 mL) of compound 21.2 (240 mg, 0.38 mmol) and pyridinium toluenesulfonate (10 mg) was heated at reflux for 30 min. After cooled to room temperature, the reaction mixture was concentrated under reduced pressure. The residue was partitioned between methylene chloride and saturated  $\text{NaHCO}_3$  aqueous solution, dried, concentrated under reduced pressure and purified to afford 22.1 (225 mg, 88%).

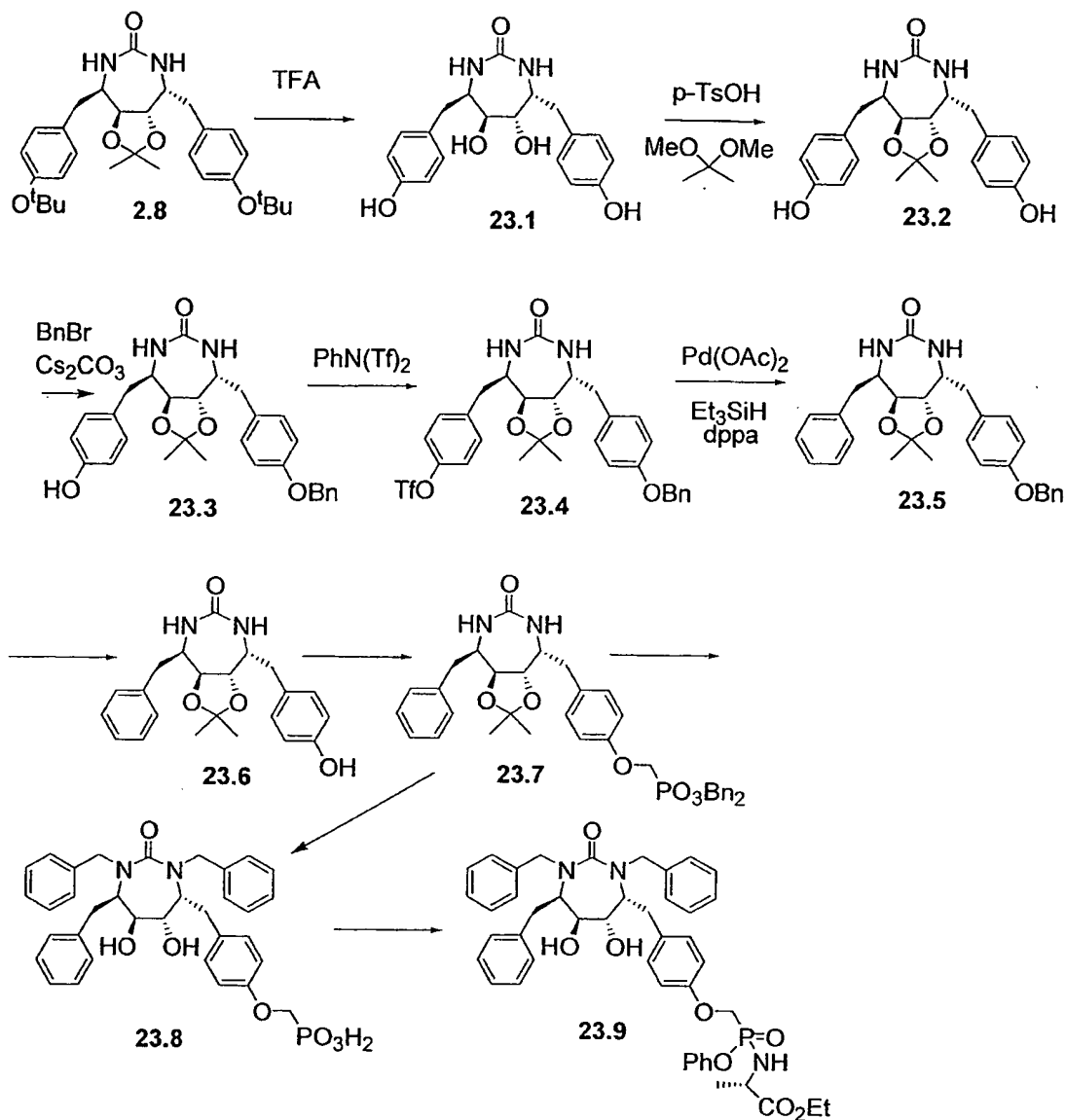
**Monomethoxy derivative 22.2:** A THF solution (10 mL) of 22.1 (225 mg, 0.33 mmol) was treated with cesium carbonate (160 mg, 0.5 mmol) and iodomethane (52 mg, 0.37 mmol) at room temperature overnight. The reaction mixture was concentrated under reduced pressure, and purified by preparative silica gel column chromatography to afford 22.2 (66 mg, 29%) and recovered starting material 22.1 (25 mg, 11%).

**Diethyl phosphonate 22.3:** A methylene chloride solution (2 mL) of 22.2 (22 mg, 32  $\mu\text{mol}$ ), DIPEA (9 mg, 66  $\mu\text{mol}$ ), and p-nitrophenyl chloroformate (8 mg, 40  $\mu\text{mol}$ ) was stirred at room temperature for 30 min. The resulting reaction mixture was reacted with DIPEA (10 mg, 77  $\mu\text{mol}$ ), and aminoethyl diethylphosphonate 14.7 (12 mg, 45  $\mu\text{mol}$ ) at room temperature overnight. The reaction mixture was washed with 5% citric acid solution, saturated  $\text{NaHCO}_3$ , dried, and purified by preparative TLC to afford 22.3 (12 mg, 43%).

**Bis(3-aminobenzyl)-diethylphosphonate ester 22.5:** An ethyl acetate/t-BuOH (4 mL/2 mL) solution of **22.3** (12 mg, 13  $\mu$ mol) was hydrogenated at 1 atm in the presence of 10% Pd/C (95 mg) at room temperature for 5 h. The catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and purified by preparative TLC to give **22.4** (8 mg, 72%). A methylene chloride solution (0.5 mL) of **22.4** (8 mg) was treated with TFA (0.1 mL) at room temperature for 1 h., concentrated under reduced pressure, and then azeotroped with CH<sub>3</sub>CN twice to afford **22.5** (8.1 mg, 81%). NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD):  $\delta$  7.2 (d, 1H), 6.95-7.15 (m, 6H), 6.75-6.9 (m, 5 H), 4.66 (d, 1H), 4.46 (d, 1H), 4.06-4.15 (m, 4H), 3.75 (s, 3H), 3.6-3.7 (m, 4H), 2.6-3.1 (m, 6H), 2.0-2.1 (m, 2H), 1.30 (t, 6H). P NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): 29.53 ppm. MS: 790 (M + 1).

**Bis(3-aminobenzyl) diethylphosphonate ester 22.7:** Compound **22.7** was prepared from **22.2** (22 mg, 32  $\mu$ mol) and aminomethyl diethylphosphonate **22.8** as shown above for the preparation of **22.5** from **22.2**. NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD):  $\delta$  7.24 (d, 1H), 6.8-7.12 (m, 11H), 4.66 (d, 1H), 4.45 (d, 1H), 4.06-4.15 (m, 4H), 3.75 (s, 3H), 2.6-3.1 (m, 6H), 1.30 (t, 6H). P NMR (CDCl<sub>3</sub> + ~10 %CD<sub>3</sub>OD): 22.75 ppm. MS: 776 (M + 1).

Scheme 23



**Diol 23.1:** To a solution of compound 2.8 (2.98 g, 5.84 mmol) in methylene chloride (14 mL) was added TFA (6 mL). The resulted mixture was stirred at room temperature for 2 h. Methanol (5 mL) and additional TFA (5 mL) were added. The reaction mixture was stirred for additional 4 h and then concentrated under reduced pressure. The residue was washed with hexane/ethyl acetate (1:1) and dried to afford compound 23.1 (1.8 g, 86%) as an off-white solid.

**Benzyl ether 23.3:** To a solution of compound **23.1** (1.8 g, 5.03 mmol) in DMF (6 mL) and 2,2-dimethoxyl propane (12 mL) was added p-toluenesulfonic acid monohydrate (0.095 g, 0.5 mmol). The resultant mixture was stirred at 65°C for 3 h. The excess 2,2-dimethoxyl propane was slowly distilled. The reaction mixture was cooled to room temperature and charged with THF (50 mL), benzyl bromide (0.8 mL, 6.73 mmol) and cesium carbonate (2.0 g, 6.13 mmol). The resulted mixture was stirred at 65°C for 16 h. The reaction was quenched with acetic acid aqueous solution (4%, 100 mL) at 0°C, and extracted with ethyl acetate. The organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford desired mono protected compound **23.3** (1.21 g, 49%).

**Benzyl ether 23.5:** To a solution of compound **23.3** (0.65 g, 1.33 mmol) and N-phenyltrifluoromethanesulfonimide (0.715 g, 2 mmol) in THF (12 mL) was added cesium carbonate (0.65 g, 2 mmol). The mixture was stirred at room temperature for 3 h. The reaction mixture was filtered through a pad of silica gel and concentrated under reduced pressure. The residue was purified on silica gel chromatography to give triflate **23.4** (0.85 g). To a solution of 1,3-bis(diphenylphosphino)propane (0.275g, 0.66 mmol) in DMF (10 mL) was added palladium(II) acetate (0.15 g, 0.66 mmol) under argon. This mixture was stirred for 2 min. and then added to triflate **23.4**. After stirring for 2 min., triethylsilane was added and the resulted mixture was stirred for 1.5 h. The solvent was removed under reduced pressure and the residue was purified by chromatography on silica gel to afford compound **23.5** (0.56 g, 89%).

**Phenol 23.6:** A solution of **23.5** (0.28 g, 0.593 mmol) in ethyl acetate (5 mL) and isopropyl alcohol (5 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield **23.6** (0.22 g, 97%) as a white solid.

**Dibenzyl phosphonate 23.7:** To a solution of compound **23.6** (0.215 g, 0.563 mmol) in THF (10 mL) was added dibenzyl triflate **3.11** (0.315 g, 0.74 mmol) and cesium carbonate (0.325g, 1 mmol). The mixture was stirred at room temperature for 2 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and

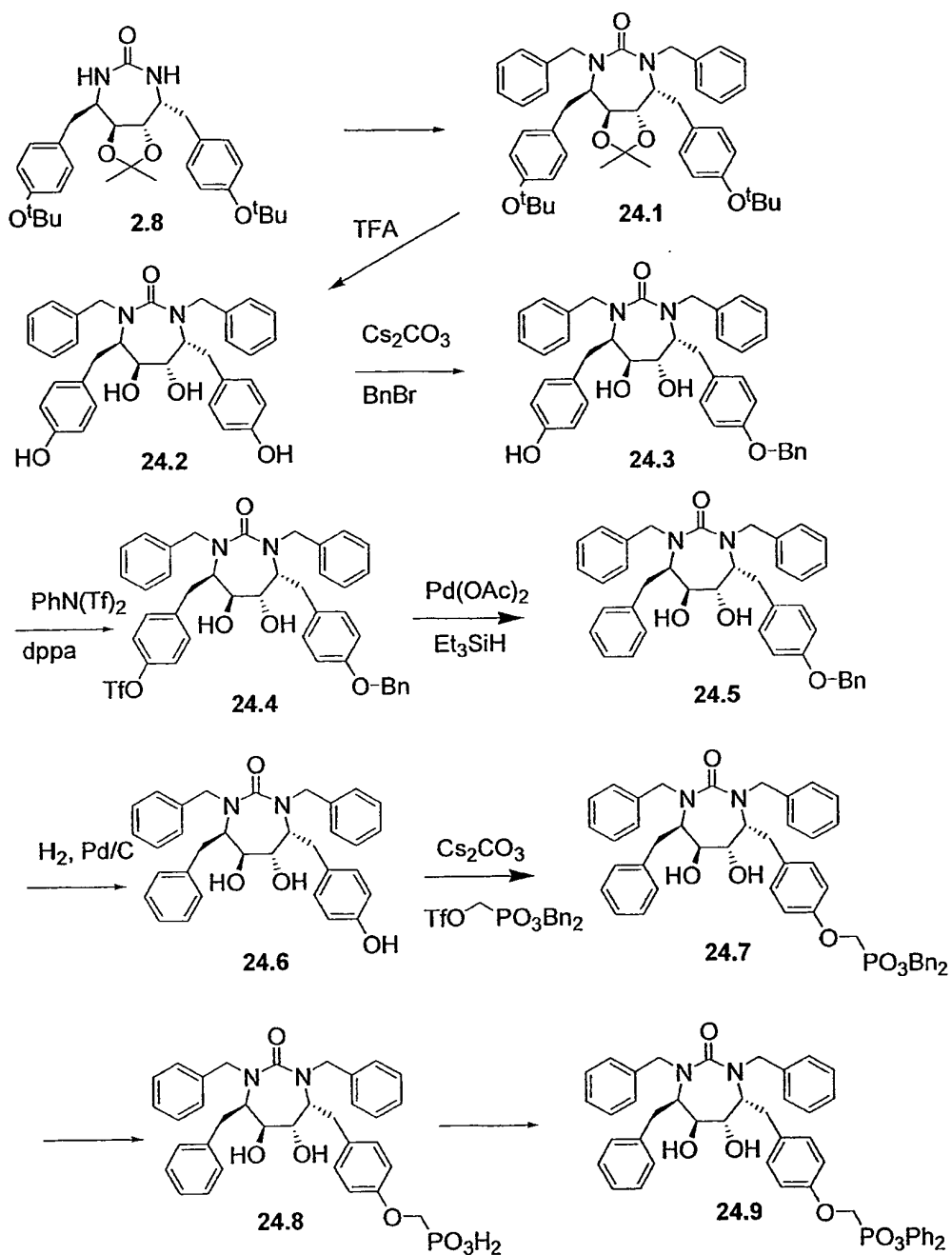


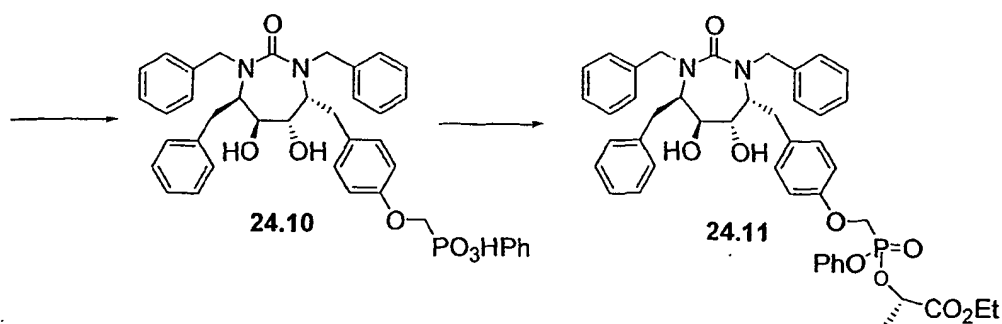
concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **23.7** (0.31 g, 84%).

**Diphenyl ester 23.8:** A solution of compound **23.7** (0.3 g, 0.457 mmol) and benzyl bromide (0.165 mL, 1.39 mmol) in THF (10 mL) was treated with potassium *tert*-butoxide (1M/THF, 1.2 mL) for 0.5 h. The mixture was diluted with ethyl acetate and washed with HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in ethyl acetate and treated with 10% Pd/C (0.05 g) under hydrogen atmosphere (balloon) for 16 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was treated with TFA (1 mL) in methanol (5 mL) for 1 h, and then concentrated under reduced pressure. The residue was dissolved in pyridine (1 mL) and mixed with phenol (0.45 g, 4.8 mmol) and 1,3-dicyclohexylcarbodiimide (0.38 g, 1.85 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound **23.8** (0.085 g, 24%).

**Mono amidate 23.9:** To a solution of **23.8** (0.085g, 0.11 mmol) in acetonitrile (1 mL) was added sodium hydroxide (1N, 0.25 mL) at 0°C. After stirred at 0°C for 1 h, the mixture was acidified with Dowex resin to pH = 3, and filtered. The filtrate was concentrated under reduced pressure. The residue was dissolved in pyridine (0.5 mL) and mixed with L-alanine ethyl ester hydrochloride (0.062 g, 0.4 mmol) and 1,3-dicyclohexyl-carbodiimide (0.125 g, 0.6 mmol). The mixture was stirred at 60°C for 0.5 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by HPLC (C-18, 65% acetonitrile / water) to afford compound **23.9** (0.02 g, 23%). <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.2 (m, 3H), 1.4 (m, 3H), 1.8 (brs, 2H), 2.8-3.1 (m, 6H), 3.5-3.7 (m, 4H), 3.78 (m, 1H), 4.0-4.18 (m, 2H), 4.2-4.4 (m, 3H), 4.9 (m, 2H), 6.8-7.4 (m, 24H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): δ 20.9, 19.8. MS: 792 (M+1).

Scheme 24





**Di-*tert* butyl ether 24.1:** To a solution of compound 2.8 (0.51 g, 1 mmol) and benzyl bromide (0.43g, 2.5 mmol) in THF (6 mL) was added potassium *tert*-butoxide (1M/THF, 2.5 mL). The mixture was stirred at room temperature for 0.5 h, then diluted with ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound 24.1 (0.62 g, 90%).

**Diol 24.2:** To a solution of compound 24.1 (0.62 g, 0.9 mmol) in methylene chloride (4 mL) was added TFA (1 mL) and water (0.1 mL). The mixture was stirred for 2 h, and then concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound 24.2 (0.443g, 92%).

**Benzyl ether 24.3:** Compound 24.3 was prepared in 46% yield according to the procedure described in Scheme 23 for the preparation of 23.3.

**Triflate 24.4:** Compound 24.4 was prepared in 95% yield according to the procedure described in Scheme 23 for the preparation of 23.4.

**Benzyl ether 24.5:** Compound 24.5 was prepared in 93% yield according to the procedure described in Scheme 23 for the preparation of 23.5.

**Phenol 24.6:** Compound 24.6 was prepared in 96% yield according to the procedure described in Scheme 23 for the preparation of 23.6 from 23.5.

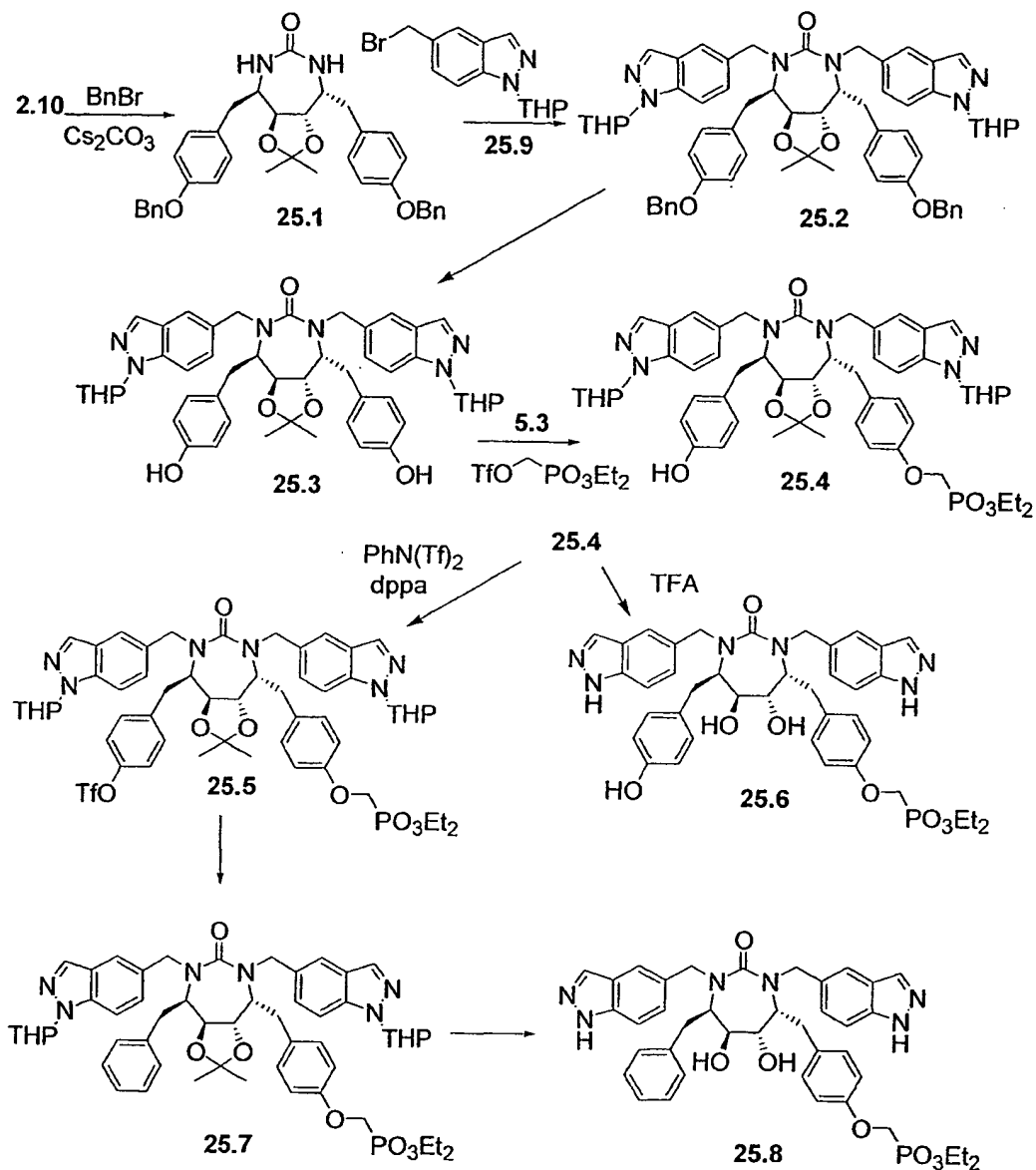
**Dibenzyl phosphonate 24.7:** Compound 24.7 was prepared in 82% yield according to the procedure described in Scheme 23 for the preparation of 23.7.

**Diacid 24.8:** A solution of 24.7 (0.16 g, 0.207 mmol) in ethyl acetate (4 mL) and isopropyl alcohol (4 mL) was treated with 10% Pd/C (0.05g) and stirred under a hydrogen atmosphere (balloon) for 4 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to yield 24.8 (0.125 g, 98%) as a white solid.

**Diphenyl ester 24.9:** To a solution of compound 24.8 (0.12 g, 0.195 mmol) in pyridine (1 mL) was added phenol (0.19 g, 2 mmol) and 1,3-dicyclohexylcarbodiimide (0.206 g, 1 mmol). The mixture was stirred at 70°C for 2 h, and then concentrated under reduced pressure. The residue was partitioned between ethyl acetate and HCl (0.2N). The organic phase was dried over magnesium sulfate, filtered and concentrated. The residue was purified by chromatography on silica gel to afford compound 24.9 (0.038 g, 25%).

**Mono lactate 24.11:** Compound 24.9 was converted, via compound 24.10, into compound 24.11 in 36% yield according to the procedure described in Scheme 23 for the preparation of 23.9 except utilizing the ethyl lactate ester in place of L-alanine ethyl ester. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.05 (t, J = 8 Hz, 1.5H), 1.1 (t, J = 8 Hz, 1.5H), 1.45 (d, J = 8 Hz, 1.5H), 1.55 (d, J = 8 Hz, 1.5H), 2.6 (brs, 2H), 2.9-3.1 (m, 6H), 3.5-3.65 (m, 4H), 4.15-4.25 (m, 2H), 4.4-4.62 (m, 2H), 4.9 (m, 2H), 5.2 (m, 1H), 6.9-7.4 (m, 24H). <sup>31</sup>P NMR (CDCl<sub>3</sub>): d 17.6, 15.5. MS: 793 (M+1).

Scheme 25



**Dibenzyl ether 25.1:** The protection reaction of compound 2.10 with benzyl bromide was carried out in the same manner as described in Scheme 23 to afford compound 25.1.

**Bis indazole 25.2:** The alkylation of compound 25.1 with bromide 25.9 was carried out in the same manner as described in Scheme 23 to afford compound 25.2 in 96% yield.

**Diol 25.3:** A solution of **25.2** (0.18 g, 0.178 mmol) in ethyl acetate (5 mL) and isopropyl alcohol (5 mL) was treated with 20% Pd(OH)<sub>2</sub>/C (0.09g) and stirred under a hydrogen atmosphere (balloon) for 24 h. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to afford **25.3** in quantitative yield.

**Diethyl phosphonate 25.4:** To a solution of compound **25.3** (0.124 g, 0.15 mmol) in acetonitrile (8 mL) and DMF (1 mL) was added potassium tert-butoxide (0.15 mL, 1M/THF). The mixture was stirred for 10 min. to form a clear solution. Diethyl triflate **5.3** (0.045 g, 0.15 mmol) was added to the reaction mixture. After stirred for 0.5 h, the reaction mixture was diluted with ethyl acetate and washed with HCl (0.1N). The organic phase was dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford compound **25.4** (0.039 g, 55% (based on recovered starting material: 0.064 g, 52%).

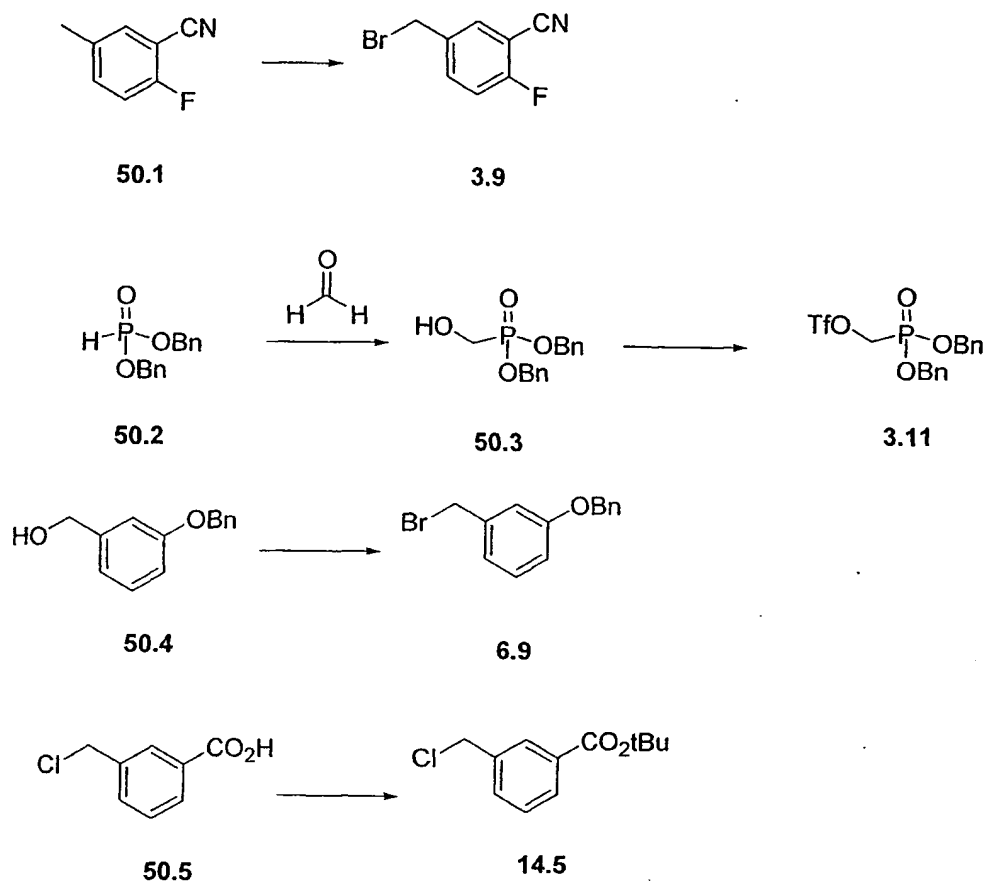
**Bisindazole 25.6:** A mixture of compound **25.4** (0.027 g), ethanol (1.5 mL), TFA (0.6 mL) and water (0.5 mL) was stirred at 60°C for 18 h. The mixture was concentrated under reduced pressure, and the residue was purified by HPLC to afford compound **25.6** as a TFA salt (0.014 g, 51%). <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.4 (t, J = 8 Hz, 6H), 2.9 (M, 4H), 3.2 (m, 2H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.75 (d, J = 9 Hz, 2H), 6.9 (m, 4H), 7.0 (d, J = 9 Hz, 2H), 7.4-7.6 (m, 6H), 8.1 (brs, 2H). <sup>31</sup>P NMR (CD<sub>3</sub>OD): δ 20.8. MS: 769 (M+1).

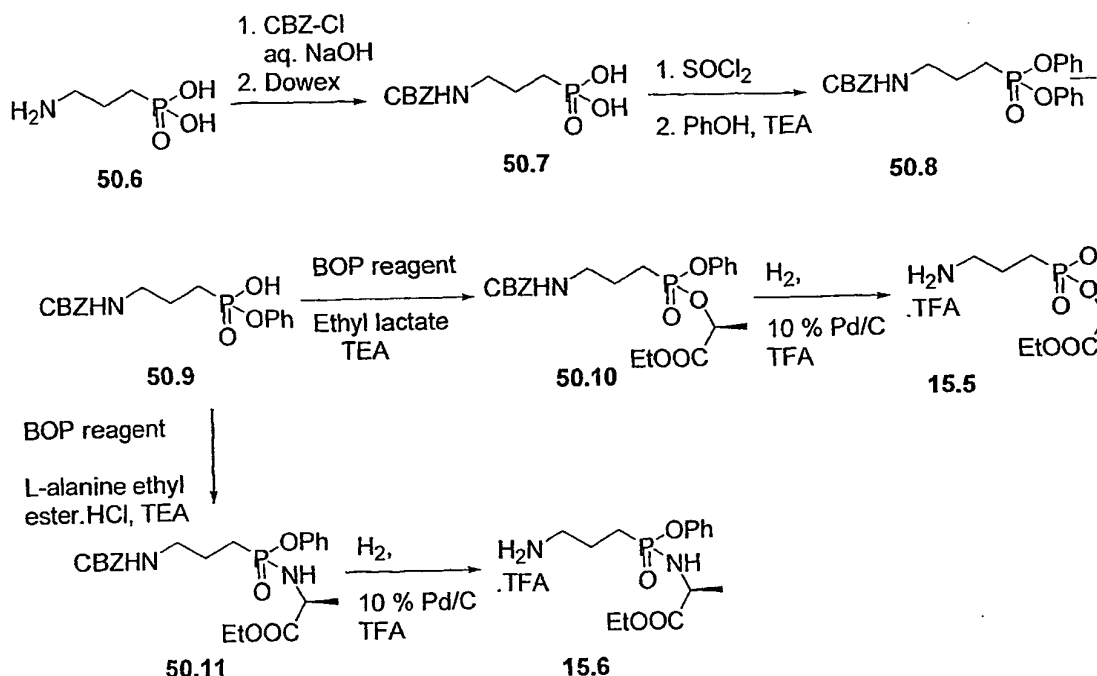
**Diethyl phosphonate 25.7:** Compound **25.4** was converted into compound **25.7** in 76% yield according to the procedures described in Scheme 23 for the conversion of **23.3** into **23.5**.

**Bis indazole 25.8:** Compound **25.7** (0.029 g) was treated in the same manner as compound **25.4** in the preparation of **25.6** to afford compound **25.8** as a TFA salt (0.0175 g, 59%). <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 1.4 (t, J = 8 Hz, 6H), 3.0 (M, 4H), 3.15 (d, J = 14 Hz, 1H), 3.25 (d, J = 14 Hz, 1H), 3.58 (brs, 2H), 3.65 (m, 2H), 4.25 (m, 4H), 4.42 (d, J = 10 Hz, 2H), 4.85 (m, 2H), 6.9 (d, J = 9 Hz, 2H), 7.0 (d, J = 9 Hz, 2H), 7.1 (d, J = 7 Hz, 2H), 7.2-7.6 (m, 9H), 8.1 (brs, 2H). <sup>31</sup>P NMR (CD<sub>3</sub>OD): δ 20.8. MS: 753 (M+1).

## Preparation of Alkylating and Phosphonate Reagents

Scheme 50





**3-cyano-4-fluoro-benzylbromide 3.9:** The commercially available 2-fluoro-4-methylbenzonitrile **50.1** (10 g, 74 mmol) was dissolved in carbon tetrachloride (50 mL) and then treated with NBS (16 g, 90 mmol) followed by AIBN (0.6 g, 3.7 mmol). The mixture was stirred at 85°C for 30 min and then allowed to cool to room temperature. The mixture was filtered and the filtrate concentrated under reduced pressure. The residue was purified by silica gel eluting with 5-20% ethyl acetate in hexanes to give **3.9** (8.8 g, 56%).

**4-benzyloxy benzyl chloride 3.10** is purchased from Aldrich

**Dibenzyl triflate 3.11:** To a solution of dibenzyl phosphite **50.2** (100 g, 381 mmol) and formaldehyde (37% in water, 65 mL, 860 mmol) in THF (200 mL) was added TEA (5 mL, 36 mmol). The resulted mixture was stirred for 1 h, and then concentrated under reduced pressure. The residue was dissolved in methylene chloride and hexane (1:1, 300 mL), dried over sodium sulfate, filtered through a pad of silica gel (600 g) and eluted with ethyl acetate and hexane (1:1). The filtrate was concentrated under reduced pressure. The residue **50.3** (95 g) was dissolved in methylene chloride (800 mL), cooled to -78°C and then charged with pyridine (53 mL, 650 mmol). To this cooled solution was slowly added trifluoromethanesulfonic anhydride (120 g, 423 mmol). The resulted reaction mixture was



stirred and gradually warmed up to  $-15^{\circ}\text{C}$  over 1.5 h period of time. The reaction mixture was cooled down to about  $-50^{\circ}\text{C}$ , diluted with hexane-ethyl acetate (2:1, 500 mL) and quenched with aqueous phosphoric acid (1M, 100 mL) at  $-10^{\circ}\text{C}$  to  $0^{\circ}\text{C}$ . The mixture diluted with hexane-ethyl acetate (2:1, 1000 mL). The organic phase was washed with water, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The residue was purified by chromatography on silica gel to afford dibenzyl triflate **3.11** (66 g, 41%) as a colorless oil.

**Diethyl triflate 5.3** is prepared as described in Tet Lett. 1986, 27, p1477-1480

**3-Benzyloxybenzylbromide 6.9:** To a solution of triphenyl phosphine (15.7 g, 60 mmol) in THF (150 mL) was added a solution of carbon tetrabromide (20 g, 60 mmol) in THF (50 mL). A precipitation was formed and stirred for 10 min. A solution of 3-benzyloxybenzyl alcohol **50.4** (10 g, 46.7 mmol) was added. After stirred for 1.5 h, the reaction mixture was filtered and concentrated under reduced pressure. The majority of triphenyl phosphine oxide was removed by precipitation from ethyl acetate-hexane. The crude product was purified by chromatography on silica gel and precipitation from hexane to give the desired product 3-Benzyloxybenzylbromide **6.9** (10 g, 77%) as a white solid.

**t-Butyl-3-chloromethyl benzoate 14.5:** A benzene solution (15 ml) of 3-chloromethylbenzoic acid **50.5** (1 g, 5.8 mmol) was heated at reflux, followed by the slow addition of N,N-dimethylformamide-di-t-butylacetal (5 m). The resulting solution was refluxed for 4 h, concentrated under reduced pressure and purified by silica gel column to afford **14.5** (0.8 g, 60 %).

**Aminopropyl-diethylphosphonate 14.6** is purchased from Acros

**Aminoethyl-diethylphosphonate oxalate 14.7** is purchased from Acros

**Aminopropyl-phenol-ethyl lactate phosphonate 15.5**

**N-CBZ-aminopropyl diphenylphosphonate 50.8:** An aqueous sodium hydroxide solution (50 mL of 1 N solution, 50 mmol) of 3-aminopropyl phosphonic acid **50.6** (3 g, 1.5 mmol)

was reacted with CBZ-Cl (4.1 g, 24 mmol) at room temperature overnight. The reaction mixture was washed with methylene chloride, acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to dryness. The crude N-CBZ-aminopropyl phosphonic acid **50.7** (5.8 mmol) was suspended in CH<sub>3</sub>CN (40 mL), and reacted with thionyl chloride (5.2 g, 44 mmol) at reflux for 4 hr, concentrated, and azeotroped with CH<sub>3</sub>CN twice. The reaction mixture was redissolved in methylene chloride (20 mL), followed by the addition of phenol (3.2 g, 23 mmol), was cooled to 0°C. To this 0°C cold solution was added TEA (2.3 g, 23 mmol), and stirred at room temperature overnight. The reaction mixture was concentrated and purified on silica gel column chromatograph to afford **50.8** (1.5 g, 62 %).

**Monophenol derivative 50.9:** A CH<sub>3</sub>CN solution (5 mL) of **50.8** (0.8 g, 1.88 mmol) was cooled to 0°C, and treated with 1N NaOH aqueous solution (4 mL, 4 mmol) for 2 h. The reaction was diluted with water, extracted with ethyl acetate, acidified with Dowex 50wx8-200. The aqueous solution was concentrated to dryness to afford **50.9** (0.56 g, 86%).

**Monolactate derivative 50.10:** A DMF solution (1 mL) of crude **50.9** (0.17 g, 0.48 mmol), BOP reagent (0.43 g, 0.97 mmol), ethyl lactate (0.12 g, 1 mmol), and DIPEA (0.31 g, 2.4 mmol) was reacted for 4 hr at room temperature. The reaction mixture was partitioned between methylene chloride and 5 % citric acid aqueous solution. The organic solution was separated, concentrated, and purified on preparative TLC to give **50.10** (0.14 g, 66%).

**3-Aminopropyl lactate phosphonate 15.5:** An ethyl acetate/ethanol solution (10 mL/2 mL) of **50.10** (0.14 g, 0.31 mmol) was hydrogenated at 1 atm in the presence of 10% Pd/C (40 mg) for 3 hr. The catalyst was filtered off. The filtrate was concentrated to dryness to afford **15.5** (0.14 g, quantitative). NMR (CDCl<sub>3</sub>): δ 8.0-8.2 (b, 3H), 7.1-7.4 (m, 5H), 4.9-5.0 (m, 1H), 4.15-4.3 (m, 2H), 3.1-3.35 (m, 2H), 2.1-2.4 (m, 4H), 1.4 (d, 3H), 1.3 (t, 3H).

**Aminopropyl-phenol-ethyl alanine phosphonate 15.6:** Compound **15.6** (80 mg) was prepared from the reaction of **50.9** (160 mg, 0.45 mmol) and L-alanine ethyl ester hydrochloride salt (0.11g, 0.68 mmol) in the presence of DIPEA and BOP reagent to give **50.11**, followed by the hydrogenation in the presence of 10% Pd/C and TFA to yield **15.6**. NMR (CDCl<sub>3</sub> + ~10 % CD<sub>3</sub>OD): δ 8.0-8.2 (b), 7.25-7.35 (t, 2H), 7.1-7.2 (m, 3H), 4.0-4.15

(m, 2H), 3.8-4.0 (m, 1H), 3.0-3.1 (m, 2H), 1.15-1.25 (m, 6H). P NMR ( $\text{CDCl}_3$  + ~10 %  $\text{CD}_3\text{OD}$ ): 32.1 & 32.4 ppm.

**Aminopropyl dibenzyl phosphonate 15.7 :**

**N-BOC-3-aminopropyl phosphonic acid 50.13:** A THF-1N aqueous solution (16 mL-16 mL) of 3-aminopropyl phosphonic acid 50.12 (1 g, 7.2 mmol) was reacted with  $(\text{BOC})_2\text{O}$  (1.7 g, 7.9 mmol) overnight at room temperature. The reaction mixture was concentrated, and partitioned between methylene chloride and water. The aqueous solution was acidified with Dowex 50wx8-200. The resin was filtered off. The filtrate was concentrated to give 50.13 (2.2 g, 92 %).

**N-BOC-3-aminopropyl dibenzyl phosphonate 50.14:** A  $\text{CH}_3\text{CN}$  solution (10 mL) of 50.13 (0.15 g, 0.63 mmol), cesium carbonate (0.61 g, 1.88 mmol), and benzyl bromide (0.24 g, 1.57 mmol) was heated at reflux overnight. The reaction mixture was cooled to room temperature, and diluted with methylene chloride. The white solid was filtered off, washed thoroughly with methylene chloride. The organic phase was concentrated, and purified on preparative TLC to give 50.14 (0.18 g, 70%). MS: 442 (M + Na).

**Aminopropyl dibenzyl phosphonate 15.7:** A methylene chloride solution (1.6 mL) of 50.14 (0.18 g) was treated with TFA (0.4 mL) for 1 hr. The reaction mixture was concentrated to dryness, and azeotroped with  $\text{CH}_3\text{CN}$  twice to afford 15.7 (0.2 g, as TFA salt). NMR ( $\text{CDCl}_3$ ):  $\delta$  8.6 (b, 2H), 7.9 (b, 2H), 7.2-7.4 (m, 10H), 4.71-5.0 (2 abq, 4H), 3.0 (b, 2H), 1.8-2 (m, 4H).  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ): 32.0 ppm. F NMR ( $\text{CDCl}_3$ ): -76.5 ppm.

**Aminomethyl diethylphosphonate 22.8** is purchased from Acros

**Bromomethyl, tetrahydropyran indazole 25.9** is prepared according to J. Org. Chem. 1997, 62, p5627

### Activity of the CCPPI Compounds

The enzyme inhibitory potency ( $K_i$ ), antiviral activity ( $EC_{50}$ ), and cytotoxicity ( $CC_{50}$ ) of the tested compounds were measured and demonstrated.

### Biological assays used for the characterization of PI prodrugs

#### HIV-1 Protease Enzyme Assay ( $K_i$ )

The assay is based on the fluorimetric detection of synthetic hexapeptide substrate cleavage by HIV-1 protease in a defined reaction buffer as initially described by M.V.Toth and G.R.Marshall, Int. J. Peptide Protein Res. 36, 544 (1990)

*Substrate:* (2-aminobenzoyl)Thr-Ile-Nle-(p-nitro)Phe-Gln-Arg

Substrate supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-2992)

*Enzyme:* recombinant HIV-1 protease expressed in E.Coli

Enzyme supplied by Bachem California, Inc. (Torrance, CA; Cat. no. H-9040)

*Reaction buffer:*

- 100 mM ammonium acetate, pH 5.3
- 1 M sodium chloride
- 1 mM ethylenediaminetetraacetic acid
- 1 mM dithiothreitol
- 10% dimethylsulfoxide

#### *Assay protocol for the determination of inhibition constant $K_i$ :*

1. Prepare series of solutions containing identical amount of the enzyme (1 to 2.5 nM) and a tested inhibitor at different concentrations in the reaction buffer
2. Transfer the solutions (190  $\mu$ L each) into a white 96-well plate
3. Preincubate for 15 min at 37°C
4. Solubilize the substrate in 100% dimethylsulfoxide at a concentration of 800  $\mu$ M. Start the reaction by adding 10  $\mu$ L of 800  $\mu$ M substrate into each well (final substrate concentration of 40  $\mu$ M)
5. Measure the real-time reaction kinetics at 37°C by using Gemini 96-well plate fluorimeter (Molecular Devices, Sunnyvale, CA) at  $\lambda(\text{Ex}) = 330$  nm and  $\lambda(\text{Em}) = 420$  nm

6. Determine initial velocities of the reactions with different inhibitor concentrations and calculate  $K_i$  (in picomolar concentration units) value by using EnzFitter program (Biosoft, Cambridge, U.K.) according to an algorithm for tight-binding competitive inhibition described by Ermoloeff J., Lin X., and Tang J., Biochemistry 36, 12364 (1997)

#### Anti-HIV-1 Cell Culture Assay ( $EC_{50}$ )

The assay is based on quantification of the HIV-1-associated cytopathic effect by a colorimetric detection of the viability of virus-infected cells in the presence or absence of tested inhibitors. The HIV-1-induced cell death is determined using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) which is converted only by intact cells into a product with specific absorption characteristics as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).

#### *Assay protocol for determination of $EC_{50}$ :*

1. Maintain MT2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Infect the cells with the wild-type HIV-1 strain IIIB (Advanced Biotechnologies, Columbia, MD) for 3 hours at 37°C using the virus inoculum corresponding to a multiplicity of infection equal to 0.01.
3. Prepare a set of solutions containing various concentrations of the tested inhibitor by making 5-fold serial dilutions in 96-well plate (100  $\mu$ L/well). Distribute the infected cells into the 96-well plate (20,000 cells in 100  $\mu$ L/well). Include samples with untreated infected and untreated mock-infected control cells.
4. Incubate the cells for 5 days at 37°C.
5. Prepare XTT solution (6 mL per assay plate) at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in water-bath for 5 min at 55°C. Add 50  $\mu$ L of N-methylphenazonium methasulfate (5  $\mu$ g/mL) per 6 mL of XTT solution.
6. Remove 100  $\mu$ L media from each well on the assay plate.
7. Add 100  $\mu$ L of the XTT substrate solution per well and incubate at 37°C for 45 to 60 min in a CO<sub>2</sub> incubator.
8. Add 20  $\mu$ L of 2% Triton X-100 per well to inactivate the virus.

9. Read the absorbance at 450 nm with subtracting off the background absorbance at 650 nm.
10. Plot the percentage absorbance relative to untreated control and estimate the  $EC_{50}$  value as drug concentration resulting in a 50% protection of the infected cells.

Cytotoxicity Cell Culture Assay ( $CC_{50}$ ):

The assay is based on the evaluation of cytotoxic effect of tested compounds using a metabolic substrate 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) as described by Weislow OS, Kiser R, Fine DL, Bader J, Shoemaker RH and Boyd MR, J. Natl. Cancer Inst. 81, 577 (1989).

*Assay protocol for determination of  $CC_{50}$ :*

1. Maintain MT-2 cells in RPMI-1640 medium supplemented with 5% fetal bovine serum and antibiotics.
2. Prepare a set of solutions containing various concentrations of the tested inhibitor by making 5-fold serial dilutions in 96-well plate (100  $\mu$ L /well). Distribute cells into the 96-well plate (20,000 cells in 100  $\mu$ L/well). Include samples with untreated cells as a control.
3. Incubate the cells for 5 days at 37°C.
4. Prepare XTT solution (6 mL per assay plate) in dark at a concentration of 2mg/mL in a phosphate-buffered saline pH 7.4. Heat the solution in a water-bath at 55°C for 5 min. Add 50  $\mu$ L of N-methylphenazonium methasulfate (5  $\mu$ g/mL) per 6 mL of XTT solution.
5. Remove 100  $\mu$ L media from each well on the assay plate and add 100  $\mu$ L of the XTT substrate solution per well. Incubate at 37°C for 45 to 60 min in a CO<sub>2</sub> incubator.
6. Add 20  $\mu$ L of 2% Triton X-100 per well to stop the metabolic conversion of XTT.
7. Read the absorbance at 450 nm with subtracting off the background at 650 nm.
8. Plot the percentage absorbance relative to untreated control and estimate the  $CC_{50}$  value as drug concentration resulting in a 50% inhibition of the cell growth. Consider the absorbance being directly proportional to the cell growth.

Resistance Evaluation (I50V and I84V/L90M fold change)

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a mutant HIV-1 strain containing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus ( $EC_{50}$ ) to a particular tested compound is measured by using the XTT-based cytopathic assay as described above. The degree of resistance to a tested compound is calculated as fold difference in  $EC_{50}$  between the wild type and a specific mutant virus. This represents a standard approach for HIV drug resistance evaluation as documented in various publications (e.g. Maguire et al., *Antimicrob. Agents Chemother.* 46: 731, 2002; Gong et al., *Antimicrob. Agents Chemother.* 44: 2319, 2000; Vandamme and De Clercq, in *Antiviral Therapy* (Ed. E. De Clercq), pp. 243, ASM Press, Washington, DC, 2001).

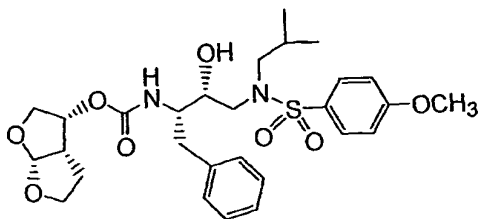
HIV-1 strains used for the resistance evaluation:

Two strains of mutant viruses containing I50V mutation in the protease gene have been used in the resistance assays: one with M46I/I47V/I50V mutations (designated I50V #1) and the other with L10I/M46I/I50V (designated I50V #2) mutations in the viral protease gene. A third virus with I84V/L90M mutations was also employed in the resistance assays. Mutants I50V #1 and I84V/L90M were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment of mutated viral protease gene that has been generated by PCR amplification. An approach similar to that described by Shi and Mellors in *Antimicrob. Agents Chemother.* 41: 2781-85, 1997 was used for the construction of mutant viruses from the generated DNA fragments. Mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After verification of protease gene sequence and determination of the infectious virus titer, the viral stock was used for drug resistance studies. Mutant I50V #2 is an amprenavir-resistant HIV-1 strain selected *in vitro* from the wild-type IIIB strain in the presence of increasing concentration of

amprenavir over a period of > 9 months using an approach similar to that described by Partaledis et al., J. Virol. 69: 5228-5235, 1995. Virus capable of growing in the presence of 5  $\mu$ M amprenavir was harvested from the supernatant of infected cells and used for resistance assays following the titration and protease gene sequencing.

Example 37: Activity of the Tested Compounds

The enzyme inhibitory potency ( $K_i$ ), antiviral activity ( $EC_{50}$ ), and cytotoxicity ( $CC_{50}$ ) of the tested compounds are summarized in Table 1.



94-003



**Table 1: Enzyme inhibition activity (Ki), antiviral cell culture activity (EC50), and cytotoxicity (CC50) of the tested compounds.**

| Substitution of (P1)phenyl | Compound   | Phosphonate substitution | HIV-1 protease inhibition Ki [pM] | Anti-HIV-1 Cell Culture Activity EC50 [nM] | Cytotoxicity CC50 [ $\mu$ M] |
|----------------------------|------------|--------------------------|-----------------------------------|--------------------------------------------|------------------------------|
| none                       | Amprenavir | none                     | 45.6 $\pm$ 18.2                   | 16 $\pm$ 2.2                               |                              |
| none                       | 94-003     | none                     | 1.46 $\pm$ 0.58                   | 1.4 $\pm$ 0.3                              |                              |
| phosphonyl                 | 27         | diacid                   | 11.8 $\pm$ 6.0                    | > 100,000                                  | > 100                        |
|                            | 28         | diethyl                  | 1.2 $\pm$ 0.8                     | 5.0 $\pm$ 2.8                              | 70                           |
| phosphonyl methoxy         | 11         | diacid                   | 2.1 $\pm$ 0.2                     | 4,800 $\pm$ 1,800                          | > 100                        |
|                            | 13         | diethyl                  | 2.6 $\pm$ 1.5                     | 3.0 $\pm$ 0                                | 50                           |
|                            | 14         | dibenzyl                 | 12.7 $\pm$ 1.9                    | 2.3 $\pm$ 0.4                              | 35                           |
|                            | 16c        | bis(Ala-ethylester)      | 15.4 $\pm$ 0.85                   | 105 $\pm$ 43                               | 60                           |
|                            | 16d        | bis(Ala-butylester)      | 18.75 $\pm$ 3.04                  | 6.0 $\pm$ 1.4                              |                              |
|                            | 16e        | bis(ABA-ethylester)      | 8.8 $\pm$ 1.7                     | 12.5 $\pm$ 3.5                             |                              |
|                            | 16f        | bis(ABA-butylester)      | 3.5 $\pm$ 1.4                     | 4.8 $\pm$ 1.8                              |                              |
|                            | 16a        | bis(Gly-ethylester)      | 29 $\pm$ 8.2                      | 330 $\pm$ 230                              |                              |
|                            | 16b        | bis(Gly-butylester)      | 4.9 $\pm$ 1.8                     | 17.5 $\pm$ 10.5                            |                              |
|                            | 16g        | bis(Leu-ethylester)      | 29 $\pm$ 9                        | 6.8 $\pm$ 0.4                              |                              |
|                            | 16h        | bis(Leu-butylester)      | 31.7 $\pm$ 19.3                   | 120 $\pm$ 42                               |                              |
|                            | 16i        | bis(Phe-ethylester)      |                                   | 17 $\pm$ 12                                |                              |
|                            | 16j        | bis(Phe-butylester)      |                                   | 35 $\pm$ 7                                 |                              |
|                            | 15         | bis(POC)                 | 36                                | 825 $\pm$ 106                              |                              |
|                            | 11         | Monoethyl, monoacid      | 0.45 $\pm$ 0.15                   | 700 $\pm$ 0                                |                              |

Cross-Resistance Profile Assay

The assay is based on the determination of a difference in the susceptibility to a particular HIV protease inhibitor between the wild-type HIV-1 strain and a recombinant HIV-1 strain expressing specific drug resistance-associated mutation(s) in the viral protease gene. The absolute susceptibility of each virus to a particular tested compound is measured by using the XTT-based cytopathic assay as described in Example B. The degree of resistance to a tested compound is calculated as fold difference in EC50 between the wild type and a specific mutant virus.

Recombinant HIV-1 strains with resistance mutations in the protease gene:

One mutant virus (82T/84V) was obtained from NIH AIDS Research and Reference Reagent Program (Rockville, MD). Majority of the mutant HIV-1 strains were constructed by a homologous recombination between three overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with the protease and reverse transcriptase genes deleted, 2. DNA fragment generated by PCR amplification containing reverse transcriptase gene from HXB2D strain (wild-type), 3. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations selected during antiretroviral therapy with various protease inhibitors. Additional mutant HIV-1 strains were constructed by a modified procedure relying on a homologous recombination of only two overlapping DNA fragments: 1. linearized plasmid containing wild-type HIV-1 proviral DNA (strain HXB2D) with only the protease gene deleted, and 2. DNA fragment generated by RT-PCR amplification from patients plasma samples containing viral protease gene with specific mutations. In both cases, mixture of DNA fragments was delivered into Sup-T1 cells by using a standard electroporation technique. The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics until the recombinant virus emerged (usually 10 to 15 days following the electroporation). Cell culture supernatant containing the recombinant virus was harvested and stored in aliquots. After determination of the virus titer the virus stock was used for drug resistance studies.

Example 39: Cross-Resistance Profile of the Tested Compounds

Cross-resistance profile of currently used HIV-1 protease inhibitors was compared with that of the newly invented compounds (Table 2).

Table 2. Cross-resistance profile of HIV-1 protease inhibitors

| Compound   | EC<br>50<br>[nM] | Fold Change in EC <sub>50</sub> Relative to WT HIV-1 |            |                          |                   |                          |                          |                   |                                 |                                 |                          |                                 | Total<br>No. of<br>Resis-<br>tant<br>Viruses <sup>b</sup> |
|------------|------------------|------------------------------------------------------|------------|--------------------------|-------------------|--------------------------|--------------------------|-------------------|---------------------------------|---------------------------------|--------------------------|---------------------------------|-----------------------------------------------------------|
|            |                  | 8K <sup>a</sup><br>46I<br>90M                        | 46I<br>84A | 10I<br>48V<br>54V<br>82A | 46I<br>47V<br>50V | 10R<br>46I<br>82T<br>84V | 30N<br>50S<br>82I<br>88D | 54V<br>71V<br>82S | 10F<br>46I<br>71V<br>82T<br>90M | 10I<br>48V<br>71V<br>82A<br>90M | 48V<br>54V<br>71V<br>82S | 10I<br>84V<br>71V<br>73S<br>90M |                                                           |
| Amprenavir | 20               | 1.25                                                 | 14         | 2                        | 38                | 4                        | 0.8                      | 4                 | 13                              | 2.5                             | 2                        | 10                              | 4                                                         |
| Nelfinavir | 14               | 13                                                   | 11         | 11.5                     | 2                 | 3                        | 43                       | 12                | 33                              | 27                              | 12                       | 65                              | 9                                                         |
| Indinavir  | 15               | 4                                                    | 10         | 15                       | nd                | 7                        | 1                        | 10                | 13                              | 28                              | 23                       | 43                              | 8                                                         |
| Ritonavir  | 15               | 34                                                   | 18         | 20                       | 13                | 47                       | 2                        | 20                | 32                              | 22                              | >50                      | 42                              | 10                                                        |
| Saquinavir | 4                | 1                                                    | 2.5        | 11                       | 1                 | 2.5                      | 1                        | 3                 | 2.5                             | 12                              | 45                       | 40                              | 4                                                         |
| Lopinavir  | 8                | nd                                                   | 9          | nd                       | 19                | 11                       | nd                       | nd                | 7.5                             | 4.5                             | 60                       | 11                              | 6                                                         |
| Tipranavir | 80               | nd                                                   | 1          | 0.4                      | 0.5               | 5                        | 0.5                      | 3.5               | 3                               | 0.3                             | 2                        | nd                              | 1                                                         |
| 94-003     | 0.5              | nd                                                   | 8          | 0.5                      | 29                | nd                       | 0.4                      | 3.5               | nd                              | nd                              | nd                       | 8                               | 3                                                         |
| GS 16503   | 16               | 1.2                                                  | 1          | 0.4                      | 3.3               | 1                        | 0.6                      | 0.9               | 1                               | 0.4                             | 0.5                      | 2                               | 0                                                         |
| GS 16571   | 22               | 1.8                                                  | 1          | 0.3                      | 0.8               | 0.6                      | 0.7                      | 0.6               | 0.8                             | 0.2                             | 0.2                      | 0.9                             | 0                                                         |
| GS 16587   | 15               | 1.5                                                  | 1          | 0.5                      | 2                 | 1                        | 1                        | 0.9               | 1                               | 0.4                             | 0.4                      | 1                               | 0                                                         |

<sup>a</sup> Resistance-associated mutations present in the viral protease. The highlighted changes represent primary resistance mutations.

<sup>b</sup> Resistance is considered as a 5-fold and higher change in the EC<sub>50</sub> value of the mutant virus relative to the wild-type virus.

**Example Section N****Plasma and PBMC Exposure Following Intravenous and Oral Administration of Prodrug to Beagle Dogs**

The pharmacokinetics of a phosphonate prodrug GS77366 (P1-monoLac-iPr), its active metabolite (metabolite X, or GS77568), and GS8373 were studied in dogs following intravenous and oral administration of the prodrug.

**Dose Administration and Sample Collection.** The in-life phase of this study was conducted in accordance with the USDA Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and followed the standards for animal husbandry and care found in the Guide for the Care and Use of Laboratory Animals, 7<sup>th</sup> Edition, Revised 1996. All animal housing and study procedures involving live animals were carried out at a facility which had been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care - International (AAALAC).

Each animal in a group of 4 female beagle dogs was given a bolus dose of GS77366 (P1-monoLac-iPr) intravenously at 1 mg/kg in a formulation containing 40% PEG 300, 20% propylene glycol and 40% of 5% dextrose. Another group of 4 female beagle dogs was dosed with GS77366 via oral gavage at 20 mg/kg in a formulation containing 60% Vitamin-E TPGS, 30% PEG 400 and 10% propylene glycol.

Blood samples were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-dose. Plasma (0.5 to 1 mL) was prepared from each sample and kept at -70°C until analysis. Blood samples (8 mL) were also collected from each dog at 2, 8 and 24 hr post dose in Becton-Dickinson CPT vacutainer tubes. PBMCs were isolated from the blood by centrifugation for 15 minutes at 1500 to 1800 G. After centrifugation, the fraction containing PBMCs was transferred to a 15 mL conical centrifuge tube and the PBMCs were washed twice with phosphate buffered saline (PBS) without Ca<sup>2+</sup> and Mg<sup>2+</sup>. The final wash of the cell pellet was kept at -70°C until analysis.

**Measurement of the prodrug, metabolite X and GS8373 in plasma and PBMCs.** For plasma sample analysis, the samples were processed by a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10 µM, from

J.T. Baker) were conditioned with 200  $\mu$ L of methanol followed by 200  $\mu$ L of water. An aliquot of 200  $\mu$ L of plasma sample was applied to each cartridge, followed by two washing steps each with 200  $\mu$ L of deionized water. The compounds were eluted from the cartridges with a two-step process each with 125  $\mu$ L of methanol. Each well was added 50  $\mu$ L of water and mixed. An aliquot of 25  $\mu$ L of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5  $\mu$ m) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, pH 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, pH 4.6. The chromatography was carried out at a flow rate of 250  $\mu$ L/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure GS77366, GS8373 and Metabolite X with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for GS77366, GS8373 and GS77568 (Metabolite X) in plasma.

For PBMC sample analysis, phosphate buffered saline (PBS) was added to each PBMC pellet to bring the total sample volume to 500  $\mu$ L in each sample. An aliquot of 150  $\mu$ L from each PBMC sample was mixed with an equal volume of methanol, followed by the addition of 700  $\mu$ L of 1% formic acid in water. The resulting mixture was applied to a Speedisk C18 solid phase extraction cartridge (1 mL, 20 mg, 10  $\mu$ m, from J.T. Baker) which had been conditioned as described above. The compounds were eluted with methanol after washing the cartridge 3 times with 10% methanol. The solvent was evaporated under a stream of N<sub>2</sub>, and the sample was reconstituted in 150  $\mu$ L of 30% methanol. An aliquot of 75  $\mu$ L of the solution was injected for LC/MS/MS analysis. The limit of quantitation was 0.1 ng/mL in the PBMC suspension.

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were calculated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R. V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

Plasma and PBMC Concentration-time Profiles.

The concentration-time profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous dosing of GS77366 were compared at 1 mg/kg in dogs. The data demonstrate that the prodrug can effectively deliver the active components (metabolite X and GS8373) into cells that are primarily responsible for HIV replication, and that the active components in these cells had much longer half-life than in plasma.

The pharmacokinetic properties of GS77568 in PBMCs following oral administration of GS77366 in dogs are compared with that of nelfinavir and amprenavir, two marketed HIV protease inhibitors (Table 3). These data show that the active component (GS77568) from the phosphonate prodrug had sustained levels in PBMCs compared to nelfinavir and amprenavir.

**Table 3. Comparison of GS77568 with nelfinavir and amprenavir in PBMCs following oral administration in beagle dogs.**

| Compound   | Dose                | $t_{1/2}$ (hr) | AUC <sub>(2-24 hr)</sub> |
|------------|---------------------|----------------|--------------------------|
| Nelfinavir | 17.5 mg/kg          | 3.0 hr         | 33,000 nM·hr             |
| Amprenavir | 20 mg/kg            | 1.7 hr         | 102,000 nM·hr            |
| GS77568    | 20 mg/kg of GS77366 | > 20 hr        | 42,200 nM·hr             |

**Example Section O****Intracellular Metabolism/In Vitro Stability****1. Uptake and Persistence in MT2 cells, quiescent and stimulated PBMC**

The protease inhibitor (PI) phosphonate prodrugs undergo rapid cell uptake and metabolism to produce acid metabolites including the parent phosphonic acid. Due to the presence of charges, the acid metabolites are significantly more persistent in the cells than non-charged PI's. In order to estimate the relative intracellular levels of the different PI prodrugs, three compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate, monoamidate phenoxy phosphonate and monolactate phenoxy phosphonate (Figure 1) were incubated at 10  $\mu$ M for 1 hr with MT-2 cells, stimulated and quiescent peripheral blood mononuclear cells (PBMC) (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 24 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. Typically, the cell lysates were centrifuged and 100  $\mu$ L of the supernatant were mixed with 200  $\mu$ L of 7.5  $\mu$ M amprenavir (Internal Standard) in 80% acetonitrile/20% water and injected into an HPLC system (70  $\mu$ L).

**HPLC Conditions:**

Analytical Column: Prodigy ODS-3, 75 x 4.6, 3 $\mu$  + C18 guard at 40°C

Gradient:

Mobile Phase A: 20 mM ammonium acetate in 10% ACN/90% H<sub>2</sub>O

Mobile Phase B: 20 mM ammonium acetate in 70% ACN/30% H<sub>2</sub>O  
30-100%B in 4 min, 100%B for 2 min, 30%B for 2 min at 2.5 mL/min.

Run Time: 8 min

UV Detection at 245 nm

Concentrations of Intracellular metabolites were calculated based on cell volume 0.2  $\mu$ L/mLn cells for PBMC and 0.338  $\mu$ L / mLn (0.676  $\mu$ L / mL) for MT-2 cells.

Chemical Structures of Selected Protease Inhibitor Phosphonate Prodrugs and Intracellular Metabolites:

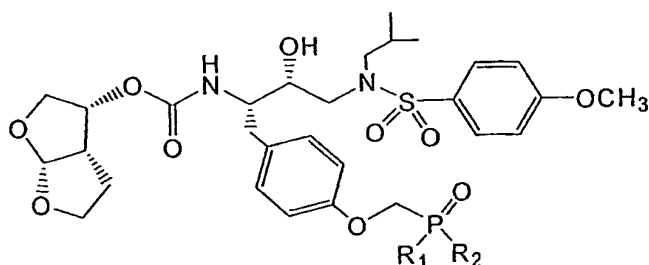


Table 4:

| GS No. | R1                          | R2                                          | EC <sub>50</sub> (nM) |
|--------|-----------------------------|---------------------------------------------|-----------------------|
| 8373   | OH                          | OH                                          | 4,800±1,800           |
| 16503  | HNCH(CH <sub>3</sub> )COOBu | HNCH(CH <sub>3</sub> )COOBu                 | 6.0±1.4               |
| 16571  | OPh                         | HNCH(CH <sub>3</sub> )COOEt                 | 15±5                  |
| 17394  | OPh                         | OCH(CH <sub>3</sub> )COOEt                  | 20±7                  |
| 16576  | OPh                         | HNCH(CH <sub>2</sub> CH <sub>3</sub> )COOEt | 12.6±4.8              |
| Met X  | OH                          | HNCH(CH <sub>3</sub> )COOH                  | >10,000               |
| Met LX | OH                          | OCH(CH <sub>3</sub> )COOEt                  | 1750±354              |

A significant uptake and conversion of all 3 compounds in all cell types was observed (Table 4). The uptake in the quiescent PBMC was 2-3-fold greater than in the stimulated cells. GS-16503 and GS-16571 were metabolized to Metabolite X and GS-8373. GS-17394 metabolized to the Metabolite LX. Apparent intracellular half-lives were similar for all metabolites in all cell types (7-12 hr). A persistence of Total Acid Metabolites of Protease Inhibitor Prodrugs in Stimulated (A), Quiescent PBMC (B) and MT-2 Cells (C) (1 hr, 10  $\mu$ M Pulse, 24 hr Chase) was observed.

## 2. Uptake and Persistence in Stimulated and Quiescent T-cells

Since HIV mainly targets T-lymphocytes, it is important to establish the uptake, metabolism and persistence of the metabolites in the human T-cells. In order to estimate the relative intracellular levels of the different PI prodrugs, GS-16503, 16571 and 17394 were incubated at 10  $\mu$ M for 1 hr with quiescent and stimulated T-cells (pulse phase). The prodrugs were compared with a non-prodrug PI, nelfinavir. After incubation, the cells were washed, resuspended in the cell culture media and incubated for 4 hr (chase phase). At specific time



points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC.

- Table 5 demonstrate the levels of total acid metabolites and corresponding prodrugs in T-cells following pulse/chase and continuous incubation. There was significant cell uptake/metabolism in T-lymphocytes. There was no apparent difference in uptake between stimulated and quiescent T-lymphocytes. There was significantly higher uptake of phosphonate PI's than nelfinavir. GS17394 demonstrates higher intracellular levels than GS16571 and GS16503. The degree of conversion to acid metabolites varied between different prodrugs. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equal mixture of the mono-phosphonic acid metabolite and GS-8373 except for GS-17394, where Metabolite LX was stable, with no GS-8373 formed.

**Table 5. Intracellular Levels of Metabolites and Intact Prodrug Following Continuous and 1 hr Pulse/4 hr Chase Incubation (10  $\mu$ M/0.7 mLn cells/1 mL) of 10  $\mu$ M PI Prodrugs and Nelfinavir with Quiescent and Stimulated T-cell**

| Compound   | Time (h) | Continuous Incubation |                    |                     |                    | 1 hr Pulse /4 hr Chase |                    |                     |                    |
|------------|----------|-----------------------|--------------------|---------------------|--------------------|------------------------|--------------------|---------------------|--------------------|
|            |          | Quiescent T-cells     |                    | Stimulated T-cells  |                    | Quiescent T-cells      |                    | Stimulated T-cells  |                    |
|            |          | Acid Met ( $\mu$ M)   | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M) | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M)    | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M) | Prodrug ( $\mu$ M) |
| 16503      | 0        | 1180                  | 42                 | 2278                | 0                  | 2989                   | 40                 | 1323                | 139                |
|            | 2        | 3170                  | 88                 | 1083                | 116                | 1867                   | 4                  | 1137                | 31                 |
|            | 4        | 5262                  | 0                  | 3198                | 31                 | 1054                   | 119                | 1008                | 0                  |
| 16571      | 0        | 388                   | 1392               | 187                 | 1417               | 1042                   | 181                | 858                 | 218                |
|            | 2        | 947                   | 841                | 1895                | 807                | 1170                   | 82                 | 1006                | 35                 |
|            | 4        | 3518                  | 464                | 6147                | 474                | 1176                   | 37                 | 616                 | 25                 |
| 17394      | 0        | 948                   | 1155               | 186                 | 1194               | 4480                   | 14                 | 2818                | 10                 |
|            | 2        | 7231                  | 413                | 3748                | 471                | 2898                   | 33                 | 1083                | 51                 |
|            | 4        | 10153                 | 167                | 3867                | 228                | 1548                   | 39                 | 943                 | 104                |
| Nelfinavir | 0        |                       | 101                |                     | 86                 |                        | 886                |                     | 1239               |
|            | 2        |                       | 856                |                     | 846                |                        | 725                |                     | 770                |
|            | 4        |                       | 992                |                     | 1526               |                        | 171                |                     | 544                |

### 3. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 $\mu$ M.

To were similar to the determine if the cell uptake/metabolism is concentration dependent, selected PI's were incubated with the 1 mL of MT-2 cell suspension (2.74 mLn cells/mL) for 1 hr at 37°C at 3 different concentrations: 10, 5 and 1  $\mu$ M. Following incubation, cells were washed twice with the cell culture medium, lysed and assayed using HPLC with UV detection. The sample preparation and analysis ones described for MT-2 cells, quiescent and stimulated PBMC. Intracellular concentrations were calculated based on cell count, a published single cell volume of 0.338 pl for MT-2 cells, and concentrations of analytes in cell lysates. Data are shown in Table 6.

Uptake of all three selected PI's in MT-2 cells appears to be concentration-independent in the 1-10  $\mu$ M range. Metabolism (conversion to acid metabolites) appeared to be concentration-dependent for GS-16503 and GS-16577 (3-fold increase at 1  $\mu$ M vs. 10  $\mu$ M) but independent for GS-17394 (monolactate). Conversion from a respective metabolite X to GS-8373 was concentration-independent for both GS-16503 and GS-16577 (no conversion was observed for metabolite LX of GS-17394).

**Table 6. Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1  $\mu$ M.**

| Compound | Extracellular Concentration, $\mu$ M | Cell-Associated Prodrug and Metabolites Concentration, $\mu$ M |        |         |       | % Conversion to acid metabolites |
|----------|--------------------------------------|----------------------------------------------------------------|--------|---------|-------|----------------------------------|
|          |                                      | Metabolite X                                                   | GS8373 | Prodrug | Total |                                  |
| GS-17394 | 10                                   | 1358                                                           | 0      | 635     | 1993  | 68                               |
|          | 5                                    | 916                                                            | 0      | 449     | 1365  | 67                               |
|          | 1                                    | 196                                                            | 0      | 63      | 260   | 76                               |
| GS-16576 | 10                                   | 478                                                            | 238    | 2519    | 3235  | 22                               |
|          | 5                                    | 250                                                            | 148    | 621     | 1043  | 40                               |
|          | 1                                    | 65                                                             | 36     | 61      | 168   | 64                               |
| GS-16503 | 10                                   | 120                                                            | 86     | 1506    | 1712  | 12                               |
|          | 5                                    | 58                                                             | 60     | 579     | 697   | 17                               |
|          | 1                                    | 12                                                             | 18     | 74      | 104   | 29                               |

\* For GS16576, Metabolite X is mono-aminobutyric acid

**4. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10  $\mu$ M.**

In order to estimate the relative intracellular levels of the different PI prodrugs under conditions simulating the in vivo environment, compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate (GS-16503), monoamidate phenoxy phosphonate (GS-16571) and monolactate phenoxy phosphonate (GS-17394) were incubated at 10  $\mu$ M for 1 hr with intact human whole blood at 37°C. After incubation, PBMC were isolated, then lysed and the lysates were analyzed by HPLC with UV detection. The results of analysis are shown in Table 7. There was significant cell uptake/metabolism following incubation in whole blood. There was no apparent difference in uptake between GS-16503 and GS-16571. GS-17394 demonstrated significantly higher intracellular levels than GS-16571 and GS-16503.

The degree of conversion to acid metabolites varies between different prodrugs after 1 hr incubation. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571 (Table 7). The metabolites, generally, were an equimolar mixture of the mono-phosphonic acid metabolite and GS-8373 (parent acid) except for GS-17394, where Metabolite LX was stable with no GS-8373 formed.

**Table 7. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10  $\mu$ M (Mean  $\pm$  SD, N=3).**

| GS#   | Intracellular Prodrug and Metabolites Concentration, $\mu$ M |                  |                | Major Intracellular Metabolites |
|-------|--------------------------------------------------------------|------------------|----------------|---------------------------------|
|       | Acid Metabolite                                              | Prodrug, $\mu$ M | Total, $\mu$ M |                                 |
| 16503 | 279 $\pm$ 47                                                 | 61 $\pm$ 40      | 340 $\pm$ 35   | X, GS-8373                      |
| 16571 | 319 $\pm$ 112                                                | 137 $\pm$ 62     | 432 $\pm$ 208  | X, GS-8373                      |
| 17394 | 629 $\pm$ 303                                                | 69 $\pm$ 85      | 698 $\pm$ 301  | LX                              |

\* PBMC Intracellular Volume = 0.2  $\mu$ L/mln

#### 5. Distribution of PI Prodrugs in PBMC

In order to compare distribution and persistence of PI phosphonate prodrugs with those of non-prodrug PI's, GS-16503, GS-17394 and nelfinavir, were incubated at 10  $\mu$ M for 1 hr with PBMC (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 20 more hr (chase phase). At specific time points, the cells were washed and lysed. The cell cytosol was separated from membranes by centrifugation at 9000 xg. Both cytosol and membranes were extracted with acetonitrile and analyzed by HPLC with UV detection.

Table 8 shows the levels of total acid metabolites and corresponding prodrugs in the cytosol and membranes before and after the 22 hr chase. Both prodrugs exhibited complete conversion to the acid metabolites (GS-8373 and X for GS-16503 and LX for GS-17394, respectively). The levels of the acid metabolites of the PI phosphonate prodrugs in the cytosol fraction were 2-3-fold greater than those in the membrane fraction after the 1 hr pulse and 10-fold greater after the 22 hr chase. Nelfinavir was present only in the membrane fractions. The uptake of GS-17394 was about 3-fold greater than that of GS-16503 and 30-fold greater than nelfinavir. The metabolites were an equimolar mixture of metabolite X and GS-8373 (parent acid) for GS-16503 and only metabolite LX for GS-17394.

**Table 8. Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following Continuous and 1 hr Pulse/22 hr Chase Incubation of 10  $\mu$ M PI Prodrugs and Nelfinavir with Quiescent PBMC.**

| GS#        | Cell Type | Fraction | Cell-Associated PI, pmol/mln cells |         |                         |         |
|------------|-----------|----------|------------------------------------|---------|-------------------------|---------|
|            |           |          | 1 hr Pulse/ 0 hr Chase             |         | 1 hr Pulse/ 22 hr Chase |         |
|            |           |          | Acid Metabolites                   | Prodrug | Acid Metabolites        | Prodrug |
| GS-16503   | PBMC      | Membrane | 228                                | 0       | 9                       | 0       |
| GS-16503   | PBMC      | Cytosol  | 390                                | 0       | 130                     | 0       |
| GS-17394   | PBMC      | Membrane | 335                                | 0       | 26                      | 0       |
| GS-17394   | PBMC      | Cytosol  | 894                                | 0       | 249                     | 0       |
| Nelfinavir | PBMC      | Membrane |                                    | 42      |                         | 25      |
| Nelfinavir | PBMC      | Cytosol  |                                    | 0       |                         | 0       |

Uptake and cell distribution of metabolites and intact prodrugs following 1 hr pulse/22 hr chase incubation of 10  $\mu$ M PI prodrugs and Nelfinavir with quiescent PBMC were measured.

#### 6. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

The *in vitro* metabolism and stability of the PI phosphonate prodrugs were determined in PBMC extract, dog plasma and human serum (Table 9). Biological samples listed below (120  $\mu$ L) were transferred into an 8-tube strip placed in the aluminum 37°C heating block/holder and incubated at 37°C for 5 min. Aliquots (2.5  $\mu$ L) of solution containing 1 mM of test compounds in DMSO, were transferred to a clean 8-tube strip, placed in the aluminum 37°C heating block/holder. 60  $\mu$ L aliquots of 80% acetonitrile/20% water containing 7.5  $\mu$ M of amprenavir as an internal standard for HPLC analysis were placed into five 8-tube strips and kept on ice/refrigerated prior to use. An enzymatic reaction was started by adding 120  $\mu$ L aliquots of a biological sample to the strip with the test compounds using a multichannel pipet. The strip was immediately vortex-mixed and the reaction mixture (20  $\mu$ L) was sampled and transferred to the Internal Standard/ACN strip. The sample was considered the time-zero sample (actual time was 1-2 min). Then, at specific time points, the

reaction mixture (20 µL) was sampled and transferred to the corresponding IS/ACN strip. Typical sampling times were 6, 20, 60 and 120 min. When all time points were sampled, an 80 µL aliquot of water was added to each tube and strips were centrifuged for 30 min at 3000xG. The supernatants were analyzed with HPLC under the following conditions:

Column: Inertsil ODS-3, 75 x 4.6 mm, 3 µm at 40°C.

Mobile Phase A: 20 mM ammonium acetate in 10%ACN/90%water

Mobile Phase B 20 mM ammonium acetate in 70%ACN/30%water

Gradient: 20% B to 100% B in 4 min, 2 min 100% B, 2 min 20% B

Flow Rate: 2 mL/min

Detection: UV at 243 nm

Run Time: 8 min

The biological samples evaluated were as follows:

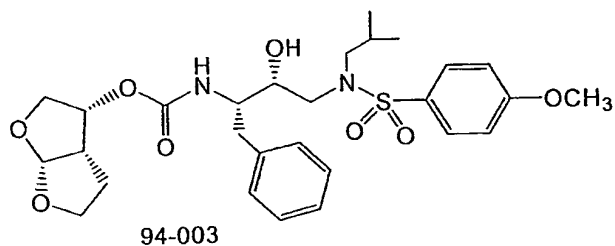
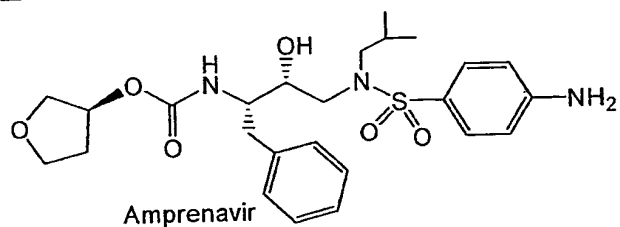
PBMC cell extract was prepared from fresh cells using a modified published procedure (A. Pompon, I. Lefebvre, J-L. Imbach, S. Kahn, and D. Farquhar, Antiviral Chemistry & Chemotherapy, 5, 91 - 98 (1994)). Briefly, the extract was prepared as following: The cells were separated from their culture medium by centrifugation (1000 g, 15 min, ambient temperature). The residue (about 100 µL,  $3.5 \times 10^8$  cells) was resuspended in 4 mL of a buffer (0.010 M HEPES, pH 7.4, 50 mM potassium chloride, 5 mM magnesium chloride and 5 mM dl-dithiothreitol) and sonicated. The lysate was centrifuged (9000 g, 10 min, 4°C) to remove membranes. The upper layer (0.5 mg protein/mL) was stored at -70°C. The reaction mixture contained the cell extract at about 0.5 mg protein/mL.

Human serum (pooled normal human serum from George King Biomedical Systems, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

Dog Plasma (pooled normal dog plasma (EDTA) from Pel Freez, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

**Table 9:** PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

| GS#   | PBMC<br>Extract <sup>1</sup><br>T <sub>1/2</sub> , min | Dog<br>Plasma<br>T <sub>1/2</sub> , min | Human<br>Serum<br>T <sub>1/2</sub> , min | HIV EC <sub>50</sub><br>(nM) |
|-------|--------------------------------------------------------|-----------------------------------------|------------------------------------------|------------------------------|
| 16503 | 2                                                      | 368                                     | >>400                                    | 6.0 ± 1.4                    |
| 16571 | 49                                                     | 126                                     | 110                                      | 15 ± 5                       |
| 17394 | 15                                                     | 144                                     | 49                                       | 20 ± 7                       |

**Example Section P****Table 10: Enzymatic and Cellular data**; **Formula II ALPPI activity****Ki [pM]**

|                  |     |
|------------------|-----|
| ≤ 10             | +++ |
| > 10 to ≤ 100    | ++  |
| > 100 to ≤ 1,000 | +   |
| > 1,000          | -   |

**EC<sub>50</sub> [nM]**

|                  |     |
|------------------|-----|
| ≤ 50             | +++ |
| > 50 to ≤ 500    | ++  |
| > 500 to ≤ 5,000 | +   |
| > 5,000          | -   |

**I50V and I84V/L90M fold change**

|              |     |
|--------------|-----|
| > 30         | +++ |
| > 10 to ≤ 30 | ++  |
| > 3 to ≤ 10  | +   |
| ≤ 3          | -   |

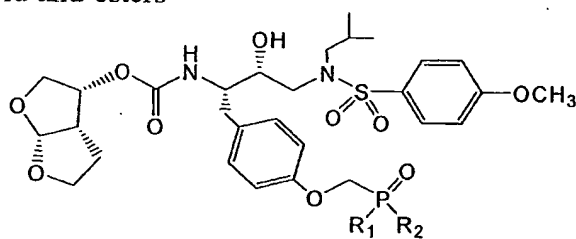
**CC<sub>50</sub> [μM]**

|             |    |
|-------------|----|
| ≤ 5         | ++ |
| > 5 to ≤ 50 | +  |
| > 50        | -  |



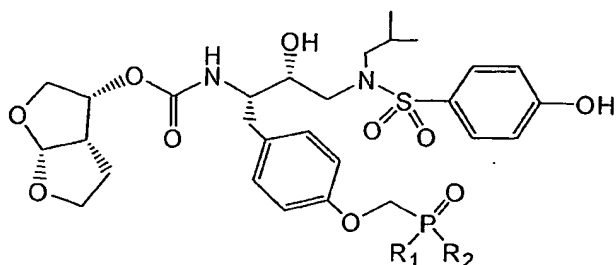
| Compound   | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I50V<br>(#2)<br>fold<br>change | I84V/L90<br>M<br>fold<br>change | CC <sub>50</sub><br>(μM) |
|------------|------------|--------------------------|--------------------------|--------------------------------|---------------------------------|--------------------------|
| Saquinavir | ++         | +++                      | —                        | —                              | +++                             |                          |
| Nelfinavir | +          | +++                      | —                        | +                              | +++                             |                          |
| Indinavir  | +          | +++                      | —                        | +                              | +++                             |                          |
| Ritonavir  | ++         | +++                      | ++                       | ++                             | +++                             |                          |
| Lopinavir  | ++         | +++                      | ++                       | +++                            | ++                              |                          |
| Amprenavir | +          | +++                      | +++                      | +++                            | ++                              | —                        |
| Atazanavir | ++         | +++                      | —                        | —                              | +++                             |                          |
| Tipranavir | ++         | ++                       | —                        | —                              | +                               |                          |
| 94-003     | +++        | +++                      | +++                      | +++                            | ++                              | +                        |
| TMC114     | +++        | +++                      | ++                       | ++                             | —                               |                          |

## P1-Phosphonic acid and esters



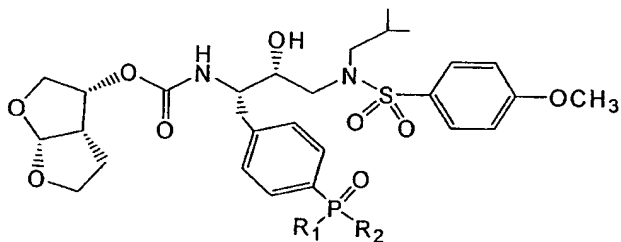
| R1                               | R2                               | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> (μM) |
|----------------------------------|----------------------------------|------------|--------------------------|--------------------------|--------------------------|-----------------------|
| OH                               | OH                               | +++        | +                        | -                        | -                        | -                     |
| OMe                              | OMe                              | ++         | +++                      |                          |                          |                       |
| OEt                              | OEt                              | +++        | +++                      | -                        | -                        | +                     |
| OCH <sub>2</sub> CF <sub>3</sub> | OCH <sub>2</sub> CF <sub>3</sub> | ++         | -                        |                          |                          |                       |
| OiPr                             | OiPr                             | ++         | +++                      | -                        | -                        |                       |
| OPh                              | OPh                              |            | +++                      |                          |                          |                       |
| OMe                              | OPh                              | ++         | +++                      |                          |                          |                       |
| OEt                              | OPh                              | +++        | +++                      |                          |                          |                       |
| OBn                              | OBn                              | ++         | +++                      | -                        | -                        | +                     |
| OEt                              | OBn                              | ++         | +++                      |                          |                          | ++                    |
| OPoc                             | OPoc                             |            | +                        |                          |                          |                       |
| OH                               | OEt                              |            | ++                       |                          |                          |                       |
| OH                               | OPh                              | +++        | -                        |                          |                          |                       |
| OH                               | OBn                              |            | +                        | -                        | -                        |                       |

## P1-Phosphonic acid and esters

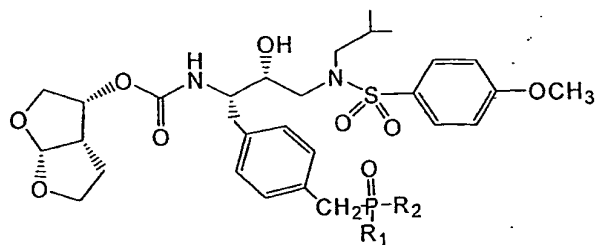


| R1 | R2 | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> (μM) |
|----|----|---------|-----------------------|-----------------------|-----------------------|-----------------------|
| OH | OH | +++     | +                     |                       |                       |                       |
| Et | Et | +++     | +++                   |                       |                       |                       |

## P1-Direct phosphonic acid and esters

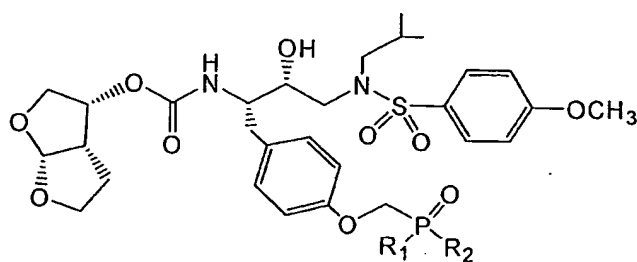


| R1  | R2  | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> μM |
|-----|-----|---------|-----------------------|-----------------------|-----------------------|---------------------|
| OH  | OH  | ++      | -                     |                       |                       |                     |
| OEt | OEt | +++     | +++                   | +                     | -                     |                     |

P1-CH<sub>2</sub>-phosphonic acid and esters

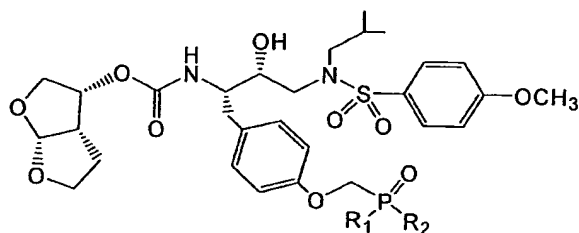
| R1 | R2 | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OE | OE | +++        | +++                      | +                        | +                        |                     |

## P1-P-Bisamidates



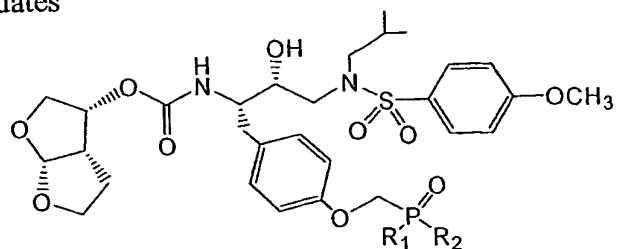
| R1     | R2     | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|--------|--------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| NHEt   | NHEt   | +++        | ++                       | —                        | —                        |                        |
| Gly-Et | Gly-Et | ++         | ++                       |                          |                          |                        |
| Gly-Bu | Gly-Bu | +++        | +++                      |                          |                          |                        |
| Ala-Et | Ala-Et | ++         | ++                       |                          | —                        | —                      |
| Ala-Bu | Ala-Bu | ++         | +++                      | +                        | —                        |                        |
| Aba-Et | Aba-Et | +++        | +++                      |                          |                          |                        |
| Aba-Bu | Aba-Bu | +++        | +++                      | ++                       | +                        |                        |
| Val-Et | Val-Et | +          | +++                      | —                        | —                        |                        |
| Leu-Et | Leu-Et | ++         | +++                      |                          |                          |                        |
| Leu-Bu | Leu-Bu | ++         | ++                       | +                        | +                        |                        |
| Phe-Et | Phe-Et |            | +++                      |                          |                          |                        |
| Phe-Bu | Phe-Bu |            | +++                      |                          |                          |                        |

## P1-P-Bislactates



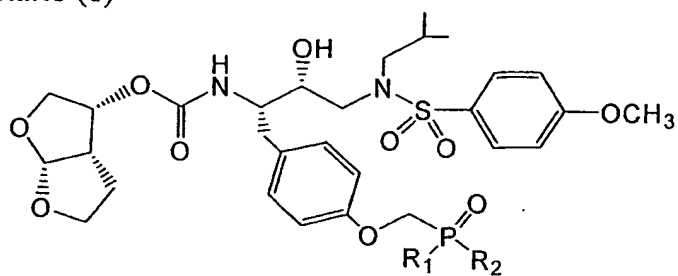
| R1      | R2      | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|---------|---------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| Glc-Et  | Glc-Et  | +++        | +                        | —                        | —                        |                        |
| Lac-Et  | Lac-Et  | ++         | ++                       | —                        | —                        |                        |
| Lac-iPr | Lac-iPr | ++         | +++                      |                          | —                        |                        |

## P1-P-Monoamidates



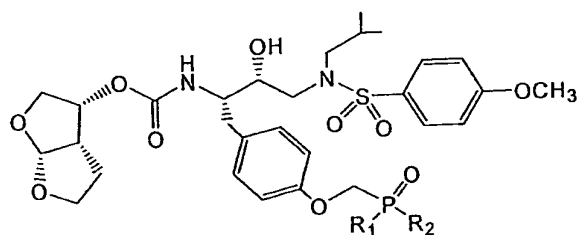
| R1  | R2         | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|-----|------------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh | Gly-Bu     | ++         | ++                       | —                        | —                        |                     |
| OPh | Ala-Me     | ++         | +++                      |                          | —                        |                     |
| OPh | Ala-Et     | +++        | +++                      | —                        | —                        |                     |
| OPh | Ala-iPr    | ++         | +++                      | —                        | —                        |                     |
| OPh | Ala-iPr    | +++        | +++                      |                          |                          |                     |
| OPh | Ala-iPr    | ++         | +++                      |                          |                          |                     |
| OPh | (D)Ala-iPr | ++         | +++                      |                          | —                        |                     |
| OPh | (D)Ala-iPr | +++        | +++                      |                          |                          |                     |
| OPh | (D)Ala-iPr | +++        | +++                      |                          |                          |                     |
| OPh | Ala-Bu     | ++         | +++                      | —                        | —                        |                     |
| OPh | Ala-Bu     | ++         | +++                      | —                        |                          |                     |
| OPh | Ala-Bu     | ++         | +++                      | —                        |                          |                     |
| OPh | Aba-Et     |            | +++                      |                          |                          |                     |
| OPh | Aba-Et     |            | +++                      | —                        | —                        |                     |
| OPh | Aba-Et     |            | ++                       |                          |                          |                     |
| OPh | Aba-Bu     |            | +++                      | +                        | —                        |                     |
| OPh | Aba-Bu     |            | ++                       | —                        | —                        |                     |
| OBn | Ala-Et     | +++        | +++                      | —                        | —                        |                     |
| OH  | Ala-OH     | +++        | —                        |                          |                          |                     |
| OH  | Ala-Bu     |            | —                        |                          |                          |                     |

## P1-P-Monolactates (1)



| R1                               | R2        | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I50V (#2)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------------------------|-----------|------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh                              | Glc-Et    | +++        | +++                      | —                        |                          | —                        |                     |
| OPh                              | Lac-Me    |            | ++                       | —                        |                          |                          |                     |
| OPh                              | Lac-Et    |            | +++                      | —                        | +                        | —                        | +                   |
| OPh                              | Lac-Et    | +++        | +++                      | —                        |                          | —                        |                     |
| OPh                              | Lac-Et    | ++         | +++                      | —                        |                          | —                        |                     |
| OPh                              | Lac-iPr   | ++         | +++                      | —                        |                          | —                        |                     |
| OPh                              | Lac-iPr   | +++        | +++                      |                          |                          |                          |                     |
| OPh                              | Lac-iPr   | ++         | +++                      |                          |                          |                          |                     |
| OPh                              | Lac-Bu    | ++         | ++                       |                          |                          | —                        |                     |
| OPh                              | Lac-Bu    | ++         | ++                       |                          |                          |                          |                     |
| OPh                              | Lac-Bu    | ++         | ++                       |                          |                          |                          |                     |
| OPh                              | Lac-EtMor |            | —                        |                          |                          |                          |                     |
| OPh                              | Lac-PrMor |            | —                        |                          |                          |                          |                     |
| OPh                              | (R)Lac-Me | +++        | +++                      |                          |                          |                          |                     |
| OPh                              | (R)Lac-Et | +++        | +++                      | —                        |                          | —                        |                     |
| OEt                              | Lac-Et    |            | ++                       |                          |                          |                          |                     |
| OCH <sub>2</sub> CF <sub>3</sub> | Lac-Et    |            | ++                       |                          |                          |                          |                     |
| OBn                              | Lac-Bn    | ++         | ++                       |                          |                          |                          |                     |
| OBn                              | (R)Lac-Bn |            |                          |                          |                          |                          |                     |
| OH                               | Lac-OH    | +++        | +                        |                          |                          | —                        |                     |
| OH                               | (R)Lac-OH | ++         | +                        |                          |                          | —                        |                     |

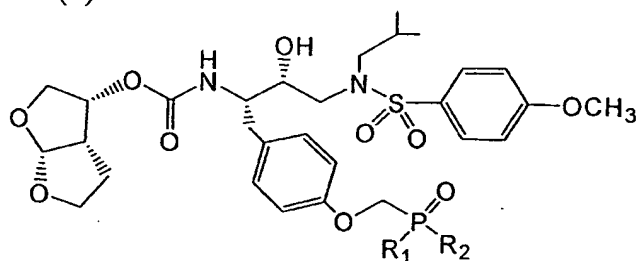
## P1-P-Monolactates (2)



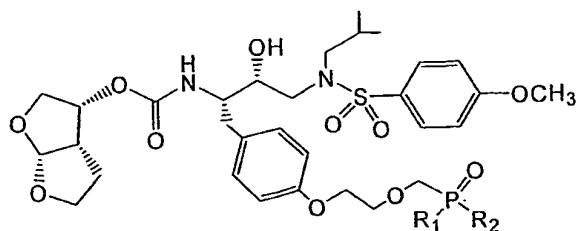
| R1  | R2           | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|-----|--------------|------------|--------------------------|--------------------------|--------------------------|------------------------|
| OPh | mix-Hba-Et   | ++         | +++                      | +                        | —                        |                        |
| OPh | (S)Hba-Et    | +          | +++                      |                          |                          |                        |
| OPh | (S)Hba-tBu   |            | +++                      |                          |                          |                        |
| OH  | (S)Hba-OH    | ++         |                          |                          |                          |                        |
| OPh | (R)Hba-Et    |            | +++                      |                          |                          |                        |
| OPh | (S)MeBut-Et  |            | +++                      |                          |                          |                        |
| OPh | (R)MeBut-Et  |            | +++                      |                          |                          |                        |
| OPh | DiMePro-Me   | ++         |                          |                          |                          |                        |
| OPh | (S)Lac-EtMor |            | —                        |                          |                          |                        |
| OPh | (S)Lac-PrMor |            | —                        |                          |                          |                        |
| OPh | (S)Lac-EtPip |            | ++                       | —                        | —                        |                        |



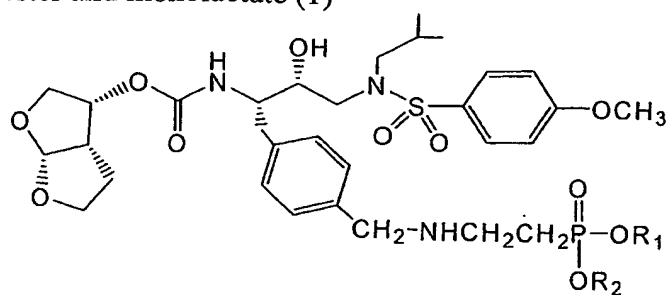
## P1-P-Monolactates (3)



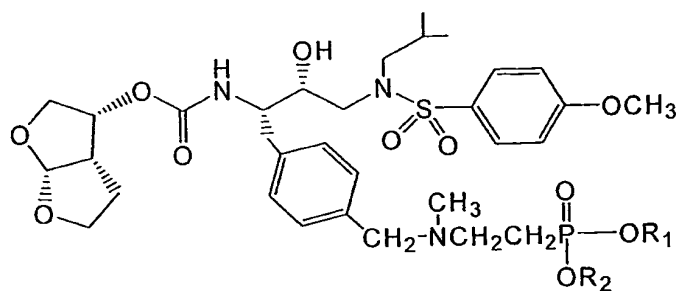
| R1                                              | R2        | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|-------------------------------------------------|-----------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh— <i>o</i> -i-But                            | (S)Lac-Et |            | +++                      |                          |                          |                     |
| OPh— <i>p</i> -n-Oct                            | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPh— <i>p</i> -n-But                            | (S)Lac-Et |            | +++                      |                          |                          |                     |
| OPh— <i>m</i> -COOBn                            | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPh— <i>m</i> -COOH                             | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPh— <i>m</i> -CH <sub>2</sub> OH               | (S)Lac-Et |            | ++                       | —                        | —                        |                     |
| OPh— <i>m</i> -CH <sub>2</sub> NH <sub>2</sub>  | (S)Lac-Et | ++         | ++                       |                          |                          |                     |
| OPh— <i>m</i> -CH <sub>2</sub> NMe <sub>2</sub> | (S)Lac-Et |            | +                        |                          |                          |                     |
| OPh— <i>m</i> -CH <sub>2</sub> Mor              | (S)Lac-Et |            | ++                       | —                        | —                        |                     |
| OPh— <i>m</i> -CH <sub>2</sub> Pip              | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPh— <i>m</i> -CH <sub>2</sub> NMeC2OM          | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPh— <i>o</i> -OEt                              | (S)Lac-Et |            | +++                      |                          |                          |                     |
| ONMe <sub>2</sub>                               | (S)Lac-Et |            | ++                       |                          |                          |                     |
| OPip                                            | (S)Lac-Et |            | +                        |                          |                          |                     |
| OMor                                            | (S)Lac-Et |            | —                        |                          |                          |                     |

P1-C<sub>2</sub>H<sub>4</sub>-P-Monolactates

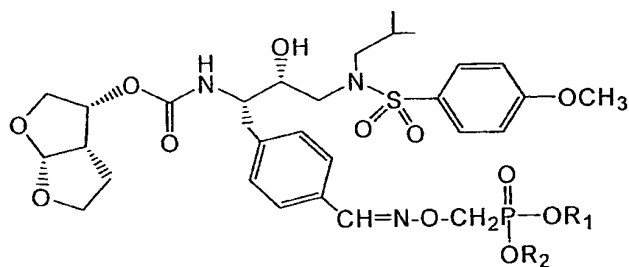
| R1                                  | R2     | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|-------------------------------------|--------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| -OC <sub>2</sub> H <sub>4</sub> OBn |        |            | +++                      |                          |                          |                     |
| OEt                                 | OEt    |            | +++                      | -                        | -                        |                     |
| OPh                                 | Lac-Et |            | ++                       | -                        | -                        |                     |
| OH                                  | OH     | ++         |                          |                          |                          |                     |
| OH                                  | Lac    | ++         |                          |                          |                          |                     |

P1-CH<sub>2</sub>N-P-diester and monolactate (1)

| R <sub>1</sub> | R <sub>2</sub> | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold<br>change | I50V (#2)<br>fold change | I84V/L9M<br>fold<br>change | CC <sub>50</sub><br>μM |
|----------------|----------------|------------|--------------------------|-----------------------------|--------------------------|----------------------------|------------------------|
| Et             | Et             | ++         | +++                      |                             | —                        |                            |                        |
| H              | H              | ++         | —                        |                             | +                        |                            |                        |
| Ph             | Lac-Et         |            | ++                       | —                           | ++                       | —                          |                        |
| Ph             | Lac-Et         |            | +                        |                             | +                        | —                          | —                      |
| Ph             | Lac-Et         |            | +                        |                             | ++                       | —                          |                        |
| Ph             | Aba-Et         |            | +                        |                             | +                        | —                          |                        |
| Ph-oEt         | Lac-Et         | ++         | ++                       | —                           | ++                       | —                          |                        |
| Ph-dM          | Lac-Et         |            | +++                      |                             | +                        | +                          |                        |
| Ph-dM          | Lac-Pr         |            | +++                      |                             |                          |                            |                        |
| H              | Lac            | ++         |                          |                             |                          |                            |                        |
| Ph             | Hba-Et         |            | ++                       |                             | ++                       | —                          |                        |
| Ph             | Hba-Et         |            | ++                       |                             | ++                       | —                          | +                      |
| Ph             | Hba-Et         |            | ++                       |                             | ++                       | —                          |                        |
| H              | Hba            | +          |                          |                             |                          |                            |                        |

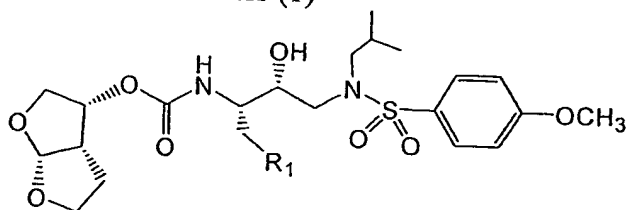
P1-CH<sub>2</sub>N-P-diester and monolactate (2)

| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| Ph             | Lac-Et         | +                      | ++                       | +                        | +                        |                        |
| H              | H              | ++                     |                          |                          |                          |                        |

P1-CH<sub>2</sub>N-P-diester and monolactate (3)

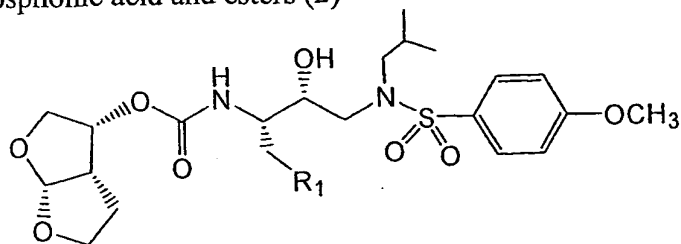
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|------------------------|
| Et             | Et             | ++                     | +++                      |                          | —                        |                        |

## P1-N-P1-Phosphonic acid and esters (1)



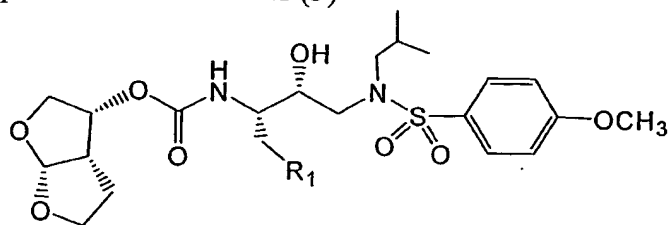
| R1 | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold<br>change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|------------|--------------------------|-----------------------------|--------------------------|---------------------|
|    | -          | ++                       |                             |                          |                     |
|    | -          | ++                       |                             |                          |                     |
|    | -          |                          |                             |                          |                     |
|    | ++         | +++                      |                             | +                        |                     |
|    |            | -                        |                             |                          |                     |
|    | -          |                          |                             |                          |                     |
|    | +          | ++                       |                             |                          |                     |
|    | ++         | +++                      |                             | +                        |                     |
|    |            | -                        |                             |                          |                     |
|    |            | -                        |                             |                          |                     |
|    | -          |                          |                             |                          |                     |
|    | +          | +++                      |                             | +                        |                     |

## P1-N-P1-Phosphonic acid and esters (2)



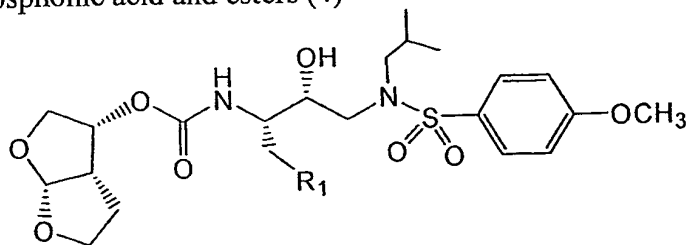
| R1 | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|    | +                      | +                        |                          | +                        |                     |
|    | ++                     | +++                      |                          | +                        |                     |
|    | ++                     | +++                      |                          |                          |                     |
|    | ++                     | ++                       |                          | -                        |                     |
|    |                        | +++                      |                          |                          |                     |
|    | ++                     | +++                      |                          | +                        |                     |
|    |                        | +++                      |                          | -                        |                     |
|    | -                      | +++                      |                          | ++                       |                     |
|    | -                      |                          |                          |                          |                     |
|    | +                      | +++                      | +++                      | -                        |                     |
|    | -                      |                          |                          |                          |                     |
|    |                        | +++                      | ++                       | +                        |                     |
|    | -                      |                          |                          |                          |                     |

## P1-N-P1-Phosphonic acid and esters (3)



| R1 | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
|    | ++         | +++                      | +                        | +                        |                     |
|    | +          | ++                       | +                        | +                        |                     |
|    | +          | ++                       | +                        | +                        |                     |
|    | +          |                          |                          |                          |                     |
|    |            |                          |                          |                          |                     |
|    | -          | -                        |                          |                          |                     |

## P1-N-P1-Phosphonic acid and esters (4)

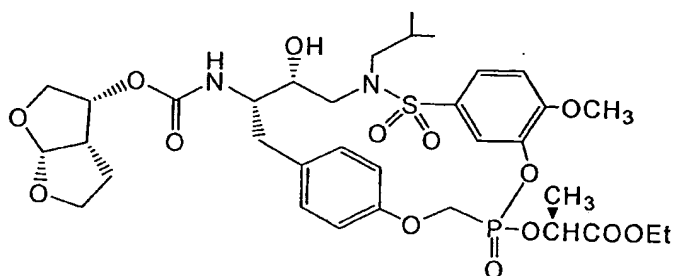


| R1 | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | 150V (#1)<br>fold change | 184V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
|    | +++        |                          |                          |                          |                     |
|    | +++        | +++                      | -                        | -                        |                     |
|    | ++         | +++                      | +                        | -                        |                     |
|    | ++         | +++                      |                          |                          |                     |
|    | ++         | ++                       |                          |                          |                     |
|    | +++        | +++                      |                          |                          |                     |
|    |            | +++                      | ++                       | -                        |                     |
|    |            | +++                      | ++                       | -                        |                     |
|    | ++         |                          |                          |                          |                     |
|    | ++         |                          |                          |                          |                     |



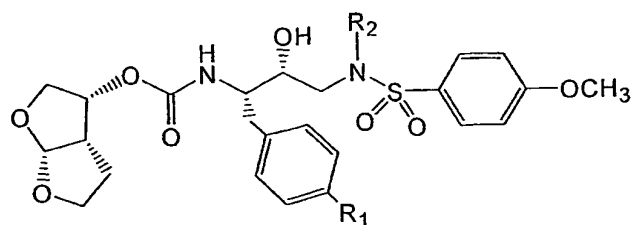
## P1- P-cyclic monolactate

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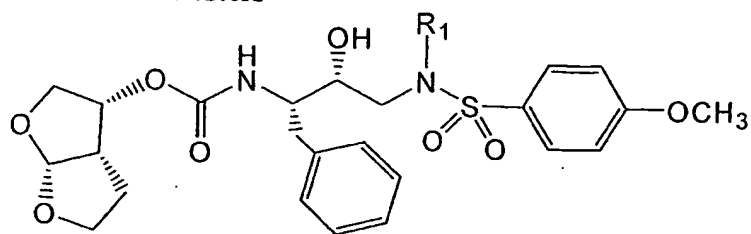
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                | nd                     | nd                       |                          |                          |                     |
|                |                | nd                     | nd                       |                          |                          |                     |

## P1'-N-P1-Phosphonic acid and esters



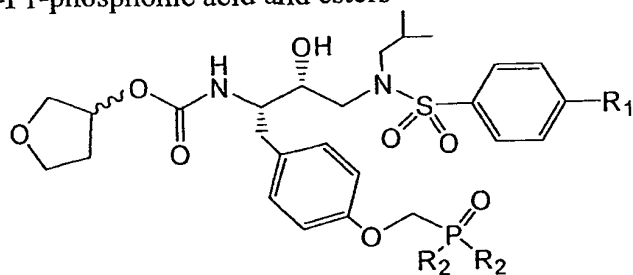
| R1                 | R2 | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> μM |
|--------------------|----|---------|-----------------------|-----------------------|-----------------------|---------------------|
| CH <sub>3</sub>    |    | ++      | +++                   | ++                    | +                     |                     |
| OH                 |    |         | +++                   | -                     | -                     |                     |
| CH <sub>2</sub> OH |    | +++     | +++                   | -                     | -                     |                     |
| OBn                |    | +++     | +++                   | -                     | -                     |                     |
| OH                 |    | -       | ++                    | -                     | -                     |                     |
| OBn                |    | -       | +++                   |                       | -                     |                     |
|                    |    | -       | -                     | +                     | +                     |                     |
|                    |    | +       | ++                    | +                     | +                     |                     |
| OH                 |    | -       | -                     |                       |                       |                     |
|                    |    | ++      | -                     |                       |                       |                     |
|                    |    | ++      | -                     |                       |                       |                     |
|                    |    | ++      | ++                    |                       |                       |                     |
|                    |    | +       | -                     |                       |                       |                     |

## P1'-Phosphonic acid and esters



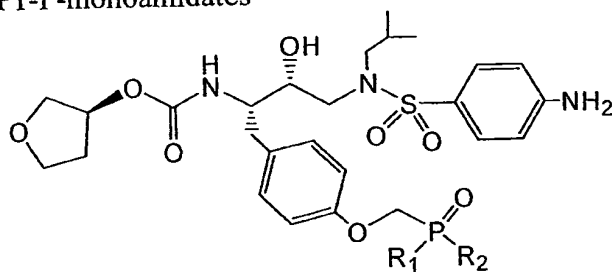
| R1 | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|------------|--------------------------|--------------------------|--------------------------|---------------------|
|    | ++         | +++                      | +++                      | +++                      |                     |
|    | +++        | +++                      | +++                      | +++                      |                     |
|    | ++         | +                        |                          | +++                      |                     |
|    | +++        | +++                      |                          | +++                      |                     |
|    | +++        | +++                      |                          | ++                       |                     |
|    | ++         | ++                       | ++                       | ++                       |                     |
|    | ++         | +++                      | +++                      | +++                      |                     |

## P2-Monofuran-P1-phosphonic acid and esters



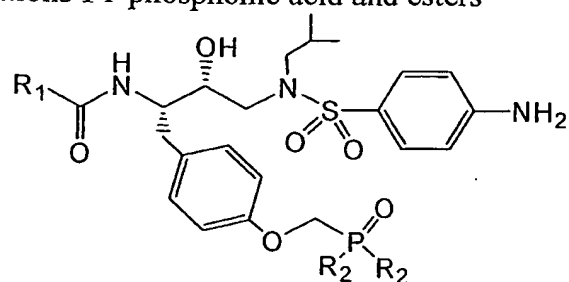
| R1              | R2     | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|-----------------|--------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OMe             | OH     |            | —                        | +++                      | +++                      |                     |
| OMe             | OEt    | +++        | +++                      | +++                      | ++                       |                     |
| OMe             | OBn    |            | +++                      | ++                       | ++                       |                     |
| OMe             | phenol | +++        | +++                      | +++                      | +                        |                     |
| OMe             | OEt    | ++         | +++                      | +++                      | ++                       |                     |
| NH <sub>2</sub> | phenol | +          | ++                       | +                        | —                        |                     |
| NH <sub>2</sub> | OH     |            | —                        |                          | +                        |                     |
| NH <sub>2</sub> | OBn    | ++         | ++                       |                          | +                        |                     |

## P2-Monofuran-P1-P-monoamidates



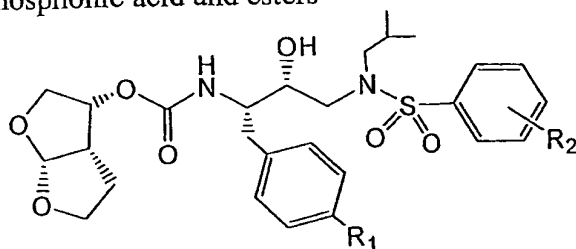
| R1  | R2      | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|-----|---------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh | Ala-iPr | ++         | ++                       |                          | +                        |                     |
| OPh | Ala-iPr | ++         | ++                       |                          |                          |                     |
| OPh | Ala-iPr | +          | ++                       |                          |                          |                     |

## P2-Other modifications-P1-phosphonic acid and esters



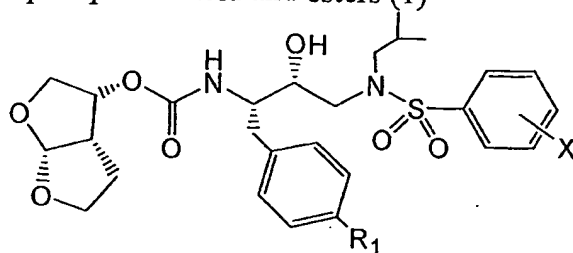
| R1 | R2     | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|--------|------------|--------------------------|--------------------------|--------------------------|---------------------|
|    | phenyl | +          | +++                      | +++                      | ++                       |                     |
|    | phenol | +          | ++                       | ++                       | +                        |                     |
|    | OH     | -          | -                        | ++                       | -                        |                     |
|    | OBn    | +          | ++                       | +                        | -                        |                     |
|    | phenyl | +          | ++                       | +++                      | +                        |                     |
|    | OH     | +          | -                        | ++                       | +                        |                     |
|    | OBn    | +          | ++                       | +++                      | +                        |                     |
|    | phenyl | -          | ++                       |                          | ++                       |                     |
|    | phenol | +          | +                        |                          | -                        |                     |
|    | OH     | +          | -                        | -                        | -                        |                     |
|    | OBn    | ++         | ++                       | +                        | -                        |                     |

## P2'-Amino-P1-phosphonic acid and esters

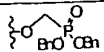
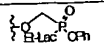
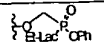


| R1 | R2                        | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----|---------------------------|------------|--------------------------|--------------------------|--------------------------|---------------------|
| OH | <i>p</i> -NH <sub>2</sub> | ++         | ++                       | —                        | —                        |                     |
|    | <i>p</i> -NH <sub>2</sub> | ++         | —                        | +                        | —                        |                     |
|    | <i>p</i> -NH <sub>2</sub> | ++         | +++                      |                          | —                        |                     |
|    | <i>p</i> -NO <sub>2</sub> | ++         | +++                      |                          | —                        |                     |
|    | <i>p</i> -NHEt            | ++         | +++                      |                          | —                        |                     |
|    | <i>p</i> -NH <sub>2</sub> | ++         | +++                      | —                        | —                        |                     |
| OH | <i>m</i> -NH <sub>2</sub> | ++         | ++                       |                          | —                        |                     |
|    | <i>m</i> -NH <sub>2</sub> | ++         | +                        |                          | —                        |                     |
|    | <i>m</i> -NH <sub>2</sub> | ++         | ++                       |                          | —                        |                     |
|    | <i>m</i> -NH <sub>2</sub> | ++         | +++                      | —                        | —                        |                     |
|    | <i>m</i> -NH <sub>2</sub> | +          | ++                       | —                        | —                        |                     |
|    | <i>m</i> -NH <sub>2</sub> | ++         | ++                       |                          |                          |                     |
|    | <i>m</i> -NH <sub>2</sub> | +          | ++                       |                          |                          |                     |

## P2'-Substituted-P1-phosphonic acid and esters (1)

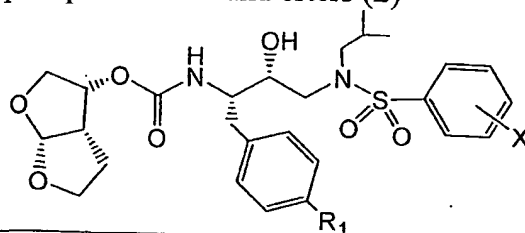


| R1 | X                          | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> μM |
|----|----------------------------|---------|-----------------------|-----------------------|-----------------------|---------------------|
|    | <i>p</i> -OH               | +++     | +                     |                       |                       |                     |
|    | <i>p</i> -OH               | +++     | +++                   |                       |                       |                     |
|    | <i>p</i> -OH               | ++      |                       |                       |                       |                     |
|    | <i>p</i> -OH               |         | +++                   |                       | -                     |                     |
|    | <i>p</i> -OBn              |         | ++                    |                       |                       |                     |
|    | <i>p</i> -OBn              |         | -                     |                       |                       |                     |
|    | <i>p</i> -H                | ++      | -                     |                       |                       |                     |
|    | <i>p</i> -H                | ++      | +++                   |                       | +                     |                     |
|    | <i>p</i> -H                |         | +++                   | +                     | +                     |                     |
|    | <i>p</i> -H                |         | ++                    |                       |                       |                     |
|    | <i>p</i> -H                | ++      |                       |                       |                       |                     |
|    | <i>p</i> -F                | ++      | +                     |                       |                       |                     |
|    | <i>p</i> -F                | ++      | +++                   |                       | +                     |                     |
|    | <i>p</i> -F                |         | +++                   | +                     | +                     |                     |
|    | <i>p</i> -F                |         | ++                    | +                     | +                     |                     |
|    | <i>p</i> -F                | ++      |                       |                       |                       |                     |
|    | <i>p</i> -CF <sub>3</sub>  | +++     | +                     |                       |                       |                     |
|    | <i>p</i> -CF <sub>3</sub>  | ++      | +++                   |                       | -                     |                     |
|    | <i>p</i> -OCF <sub>3</sub> | ++      | +                     |                       |                       |                     |
|    | <i>p</i> -OCF <sub>3</sub> | ++      | +++                   |                       | +                     |                     |

|                                                                                   |                      |    |     |  |   |  |
|-----------------------------------------------------------------------------------|----------------------|----|-----|--|---|--|
|  | <i>p</i> -CN         | ++ | +++ |  | - |  |
|  | <i>p</i> -Pip        | -  | -   |  |   |  |
|  | <i>p</i> -Pip-<br>Me | -  | -   |  |   |  |

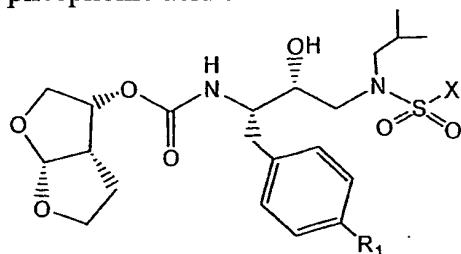


## P2'-Substituted-P1-phosphonic acid and esters (2)



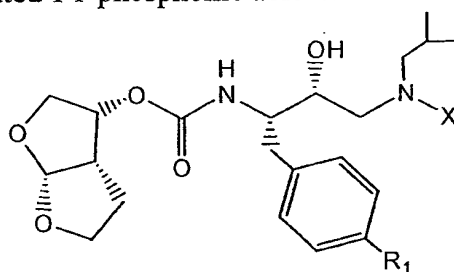
| R1                 | X                             | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|--------------------|-------------------------------|------------|--------------------------|--------------------------|--------------------------|------------------------|
|                    | <i>m</i> -Py                  | ++         | +++                      |                          |                          |                        |
|                    | <i>m</i> -Py                  | ++         |                          |                          |                          |                        |
|                    | <i>m</i> -Py                  | ++         | ++                       | +                        | -                        |                        |
|                    | <i>m</i> -Py                  | ++         | ++                       |                          |                          |                        |
|                    | <i>m</i> -Py                  | ++         |                          |                          |                          |                        |
|                    | <i>m</i> -Py-Me <sup>+</sup>  |            | +                        |                          |                          |                        |
|                    | <i>m</i> -Py-Me <sup>+</sup>  |            | ++                       |                          |                          |                        |
|                    | <i>m</i> -Py-oxide            |            | ++                       |                          |                          |                        |
|                    | <i>m</i> -Py-oxide            | ++         |                          |                          |                          |                        |
|                    | <i>m</i> -Py-oxide            | ++         | ++                       |                          | -                        |                        |
|                    | <i>m</i> -Py-oxide            | +          |                          |                          |                          |                        |
|                    | <i>m</i> -Py-oxide            |            | -                        |                          |                          |                        |
| <i>p</i> -Py-oxide | <i>p</i> -OMe                 | ++         | -                        |                          |                          |                        |
|                    | <i>p</i> -CHO                 |            | +++                      |                          |                          |                        |
|                    | <i>p</i> -CHO                 |            | +++                      |                          |                          |                        |
|                    | <i>p</i> -CH <sub>2</sub> OH  |            | +++                      | -                        | -                        |                        |
|                    | <i>p</i> -CH <sub>2</sub> OH  | ++         |                          |                          |                          |                        |
|                    | <i>p</i> -CH <sub>2</sub> OH  | ++         |                          |                          |                          |                        |
|                    | <i>p</i> -CH <sub>2</sub> Mor |            | ++                       | -                        | -                        |                        |
|                    | <i>p</i> -CH <sub>2</sub> Mor | -          |                          |                          |                          |                        |
|                    | <i>p</i> -CH <sub>2</sub> Mor | -          |                          |                          |                          |                        |

## P2'-Alkylsulfonyl-P1-phosphonic acid and esters



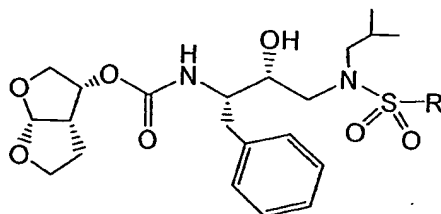
| R1 | X | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|----|---|------------|--------------------------|--------------------------|--------------------------|------------------------|
|    |   | —          | —                        |                          |                          |                        |
|    |   | +          | ++                       |                          |                          |                        |

## P2'-Carbonyl-substituted-P1-phosphonic acid and esters



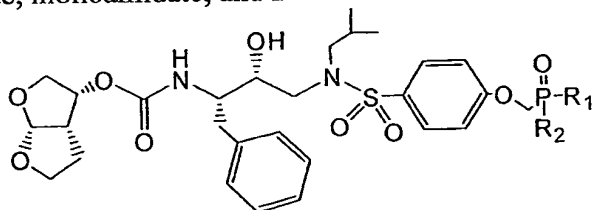
| R1 | X | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|----|---|------------|--------------------------|--------------------------|--------------------------|------------------------|
|    |   | —          |                          |                          |                          |                        |
|    |   | —          | ++                       |                          |                          |                        |
|    |   |            | +                        |                          |                          |                        |

## P2'-Phosphonic acid and esters



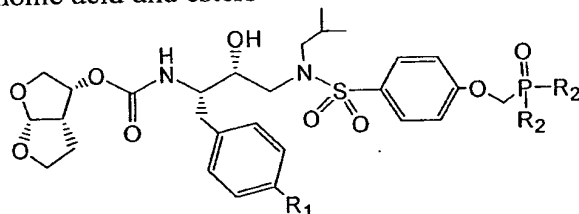
| R | Ki<br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub><br>μM |
|---|------------|--------------------------|--------------------------|--------------------------|------------------------|
|   | +++        | +++                      | -                        | -                        |                        |
|   | +++        | +                        | -                        | -                        |                        |
|   | ++         | -                        |                          |                          |                        |
|   | ++         | +++                      | ++                       | ++                       |                        |
|   | +          | ++                       | +++                      | +++                      |                        |
|   | +++        | +++                      | +                        | +                        |                        |
|   | +++        | +++                      | +++                      | ++                       |                        |
|   | ++         | ++                       | ++                       | +                        |                        |
|   | +++        | +++                      | +++                      | ++                       |                        |
|   | ++         | +++                      | ++                       | ++                       |                        |
|   | +++        | +++                      | -                        | -                        |                        |
|   | +++        | ++                       | +                        | -                        |                        |
|   | +          | ++                       | +                        | +                        |                        |
|   | -          | +                        | +++                      | ++                       |                        |
|   | +          | ++                       | +                        | -                        |                        |

## P2'-P-Bisamidate, monoamidate, and monolactate



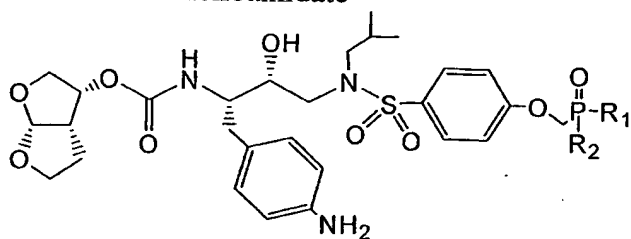
| R <sub>1</sub> | R <sub>2</sub> | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> μM |
|----------------|----------------|---------|-----------------------|-----------------------|-----------------------|---------------------|
| Ala-Bu         | Ala-Bu         | +       | ++                    | +                     | +                     |                     |
| OPh            | Ala-iPr        | ++      | ++                    |                       |                       |                     |
| OPh            | Lac-iPr        | +       | +                     |                       |                       |                     |
| OH             | Ala-OH         | ++      |                       |                       |                       |                     |

## P1-N-P2'-Phosphonic acid and esters



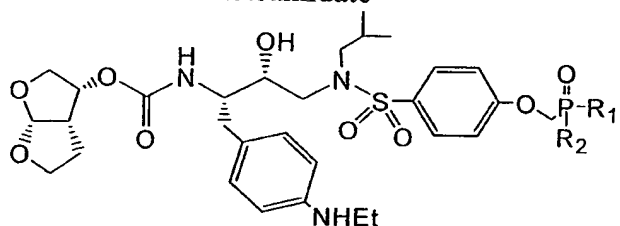
| R <sub>1</sub>                                   | R <sub>2</sub> | Ki (pM) | EC <sub>50</sub> (nM) | I50V (#1) fold change | I84V/L90M fold change | CC <sub>50</sub> μM |
|--------------------------------------------------|----------------|---------|-----------------------|-----------------------|-----------------------|---------------------|
| NO <sub>2</sub>                                  | phenol         |         | +++                   | -                     |                       |                     |
| NH <sub>2</sub>                                  | OH             | ++      | -                     |                       |                       |                     |
| NH <sub>2</sub>                                  | OEt            | +       | ++                    |                       | ++                    |                     |
| NH <sub>2</sub>                                  | OBn            | +       | +                     |                       | +                     |                     |
| NMe <sub>2</sub>                                 | OEt            | ++      | +++                   |                       | ++                    |                     |
| OH                                               | OH             | ++      | -                     |                       |                       |                     |
| OH                                               | OBn            | ++      | ++                    |                       |                       |                     |
| OC <sub>2</sub> H <sub>4</sub> NMe <sub>2</sub>  | OH             | +++     | +                     |                       |                       |                     |
| OC <sub>2</sub> H <sub>4</sub> -NMe <sub>2</sub> | OBn            | ++      | ++                    |                       |                       |                     |

## P1-N-P2'-P-Bisamidate and monoamidate



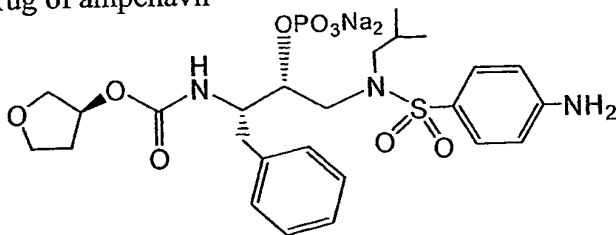
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| Ala-Bu         | Ala-Bu         | +                      | +                        |                          |                          |                     |
| OPh            | Ala-iPr        | +                      | —                        |                          |                          |                     |
| OPh            | Ala-iPr        | ++                     | —                        |                          |                          |                     |

## P1-NEt-P2'-P-Bisamidate and monoamidate



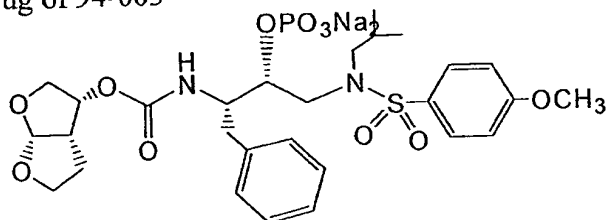
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| OPh            | Ala-iPr        | +                      | +                        |                          |                          |                     |
| OPh            | Ala-iPr        | +                      | +                        | —                        | —                        |                     |

## Phosphate prodrug of ampenavir



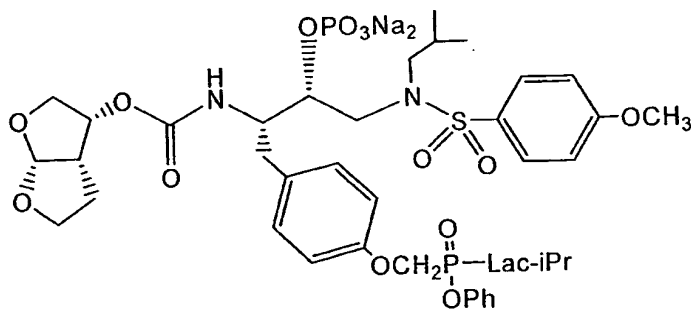
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>Fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                |                        | ++                       |                          |                          |                     |

## Phosphate prodrug of 94-003



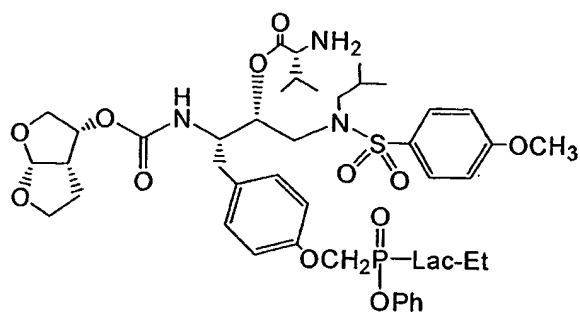
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                |                        | +++                      |                          |                          |                     |

## Phosphate prodrug of GS77366 (P1-mono(S)Lac-iPr)

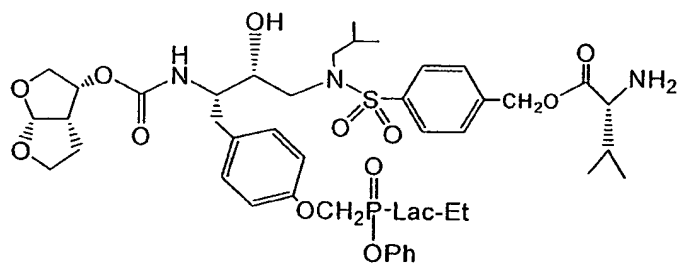


| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                |                        | +++                      |                          |                          |                     |

## Valine prodrug of (P1-mono(S)Lac-Et)



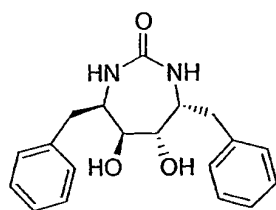
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                |                        | ++                       |                          |                          |                     |

Valine prodrug of GS278053 (P1-mono(S)Lac-Et,P2'-CH<sub>2</sub>OH)

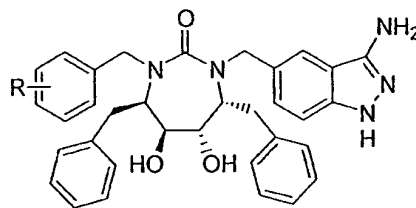
| R <sub>1</sub> | R <sub>2</sub> | K <sub>i</sub><br>(pM) | EC <sub>50</sub><br>(nM) | I50V (#1)<br>fold change | I84V/L90M<br>fold change | CC <sub>50</sub> μM |
|----------------|----------------|------------------------|--------------------------|--------------------------|--------------------------|---------------------|
|                |                |                        | ++                       |                          |                          |                     |

Table 11: Enzymatic and Cellular Activity Data

## Formula VIIIa CCLPPI activity



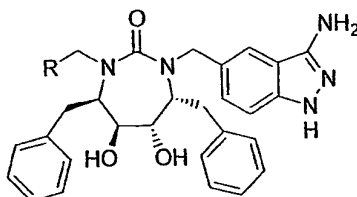
DMP-850



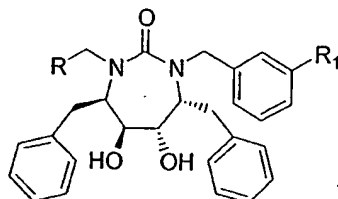
| Structure, R                                                           | Enzymatic assay        |                                |                                        | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|------------------------------------------------------------------------|------------------------|--------------------------------|----------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|                                                                        | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub> /<br>nM | 84V9<br>0M<br>IC <sub>50</sub> /<br>nM | WT                                            | 84V9<br>0M | 30N<br>82I88<br>D | 48V5<br>4V82<br>A | 48V5<br>4V82<br>S | 48V8<br>2A90<br>M | 46I50<br>V |
| H<br>(DMP-850)                                                         | 0.033                  | 3.0                            | 9.1                                    | 165                                           | 819        | 82                | 82                | 73                | 45                | 88         |
| p-OH                                                                   | 0.029                  | 3.0                            | 12                                     | 149                                           | 143        | 79                | 32                | 39                | 19                | 55         |
| p-OBn                                                                  | >5                     | 353                            | 781                                    | 2123                                          | 5312       | 1548              | ND                | ND                | ND                | ND         |
| p-OCH <sub>2</sub> PO <sub>3</sub> Bn <sub>2</sub>                     | >5                     | 276                            | 2042                                   | 2697                                          | 4963       | 2119              | ND                | ND                | ND                | ND         |
| p-OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                     | >5                     | 627                            | 1474                                   | 2480                                          | >600<br>0  | 1340              | ND                | ND                | ND                | ND         |
| p-OCH <sub>2</sub> PO <sub>3</sub> H <sub>2</sub>                      | >5                     | 551                            | 1657                                   | >1200<br>0                                    | ND         | ND                | ND                | ND                | ND                | ND         |
| m-OH                                                                   | 0.128                  | 1.6                            | 12                                     | 151                                           | 475        | 249               | 84                |                   |                   | 104        |
| m-OBn                                                                  | 0.253                  | 6.9                            | 27                                     | 218                                           | 2422       | 82                | 709               | ND                | ND                | 601        |
| m-OCH <sub>2</sub> PO <sub>3</sub> Bn <sub>2</sub><br>(N-iPr indazole) | 1.54 <sup>a</sup>      | 31                             | 72                                     | 489                                           | 514        | 237               | 159               | 171               | 168               | 708        |
| m-OCH <sub>2</sub> PO <sub>3</sub> Bn <sub>2</sub>                     | 0.177                  | 18                             | 43                                     | 898                                           | >600<br>0  | 705               | 2597              | ND                | ND                | 3121       |
| m-OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                     | 1.93 <sup>a</sup>      | 70                             | 169                                    | 665                                           | 3005       | 93                | 513               | ND                | ND                | 857        |
| m-OCH <sub>2</sub> PO <sub>3</sub> H <sub>2</sub>                      | 0.254                  | 8.3                            | 33                                     | >1200<br>0                                    | ND         | ND                | ND                | ND                | ND                | ND         |



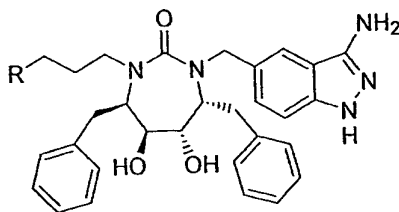
|                                                    |                    |     |     |      |           |      |      |    |    |           |
|----------------------------------------------------|--------------------|-----|-----|------|-----------|------|------|----|----|-----------|
| m-OCH <sub>2</sub> PO <sub>3</sub> Ph <sub>2</sub> | 0.543 <sub>a</sub> | 10  | 42  | 1349 | >600<br>0 | 1541 | 2183 | ND | ND | 3380      |
| m-OCH <sub>2</sub> PO <sub>3</sub> HPh             | 0.644              | 17  | 65  | 1745 | >600<br>0 | ND   | ND   | ND | ND | ND        |
| m-mono-Ala-Bu                                      | 0.858 <sub>a</sub> | 6.6 | 39  | 1042 | >600<br>0 | 425  | 790  | ND | ND | 797       |
| m-mono-Ala-Et <sup>f</sup>                         |                    | 35  | 68  | 1436 | >600<br>0 | 219  | 734  | ND | ND | 1350      |
| m-mono-Lac-Bu                                      |                    | 15  | 34  | 2663 | >600<br>0 | 1089 | ND   | ND | ND | ND        |
| m-mono-Lac-Et                                      |                    | 23  | 80  | 2609 | >600<br>0 | 516  | 5923 | ND | ND | >600<br>0 |
| m-bis-Ala-Bu                                       | 1.279 <sub>a</sub> | 18  | 103 | 1079 | >600<br>0 | 2362 | 1854 | ND | ND | 1536      |
| m-bis-Ala-Et                                       | 1.987 <sub>a</sub> | 31  | 202 | 5620 | >600<br>0 | 1852 | ND   | ND | ND | ND        |



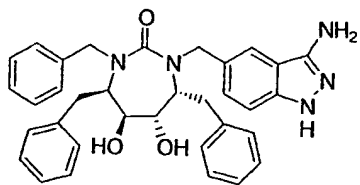
| Structure, R   | Enzymatic assay        |                              |                                        | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|----------------|------------------------|------------------------------|----------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|                | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub><br>nM | 84V90<br>M<br>IC <sub>50</sub> /<br>nM | WT                                            | 84V9<br>0M | 30N<br>82188<br>D | 48V5<br>4V82<br>A | 48V5<br>4V82<br>S | 48V8<br>2A90<br>M | 46150<br>V |
| H<br>(DMP-850) | 0.033                  | 3.0                          | 9.1                                    | 165                                           | 819        | 82                | 82                | 73                | 45                | 88         |
|                | 0.091                  | 3.4                          | 27                                     | 1548                                          | >600<br>0  | >600<br>0         | ND                | ND                | ND                | ND         |
|                | 0.354                  | 3.3                          | 25                                     | 168                                           | 909        | 750               | 277               |                   |                   | 489        |
|                | 0.157                  | 1.6                          | 10                                     | 188                                           | 476        | 666               | 240               |                   |                   | 319        |
|                | 0.044                  | 5.0                          | 27                                     | 491                                           | 387        | 234               | 238               |                   |                   | 192        |
|                | 0.362                  | 7.3                          | 70                                     | 5141                                          | >600<br>0  | 4480              | ND                | ND                | ND                | ND         |
|                | 0.112                  | 1.4                          | 6.4                                    | 603                                           | 1276       | 678               | 208               |                   |                   | 209        |
|                | <0.03                  | 1.3                          | 7.5                                    | 625                                           | 708        | 899               | 301               |                   |                   | 398        |



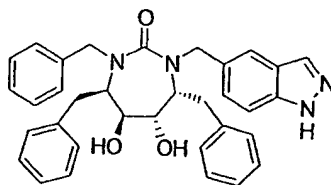
| Structure, R1                                                       | Structure, R | Enzymatic assay        |                                |                                        | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|---------------------------------------------------------------------|--------------|------------------------|--------------------------------|----------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|                                                                     |              | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub> /<br>nM | 84V<br>90M<br>IC <sub>50</sub><br>/ nM | WT                                            | 84V<br>90M | 30N<br>82I8<br>8D | 48V<br>54V<br>82A | 48V<br>54V<br>82S | 48V<br>82A<br>90M | 46I5<br>0V |
| CO <sub>2</sub> H                                                   |              |                        | 15                             | 174                                    | 3055                                          | >600<br>0  | 887               | ND                | ND                | ND                | ND         |
| CONH(CH <sub>2</sub> ) <sub>3</sub> PO <sub>3</sub> Et <sub>2</sub> |              | 0.00<br>9              | 1.1                            | 12                                     | 65                                            | 311        | 74                | 80                | 75                | 74                | 85         |
| CO <sub>2</sub> H                                                   |              |                        | 18                             | 299                                    | 2344                                          | >600<br>0  | 3360              | ND                | ND                | ND                | ND         |
| CONH(CH <sub>2</sub> ) <sub>3</sub> PO <sub>3</sub> Et <sub>2</sub> |              | <0.0<br>04             | 2.3                            | 29                                     | 176                                           | 824        | 171               | 233               | ND                | ND                | 195        |
| CO <sub>2</sub> H                                                   |              | 0.09<br>1              | 3.4                            | 27                                     | 1548                                          | >600<br>0  | >600<br>0         | ND                | ND                | ND                | ND         |
| CONH(CH <sub>2</sub> ) <sub>3</sub> PO <sub>3</sub> Et <sub>2</sub> |              | 0.15<br>7              | 1.6                            | 10                                     | 188                                           | 476        | 666               | 240               |                   |                   | 319        |



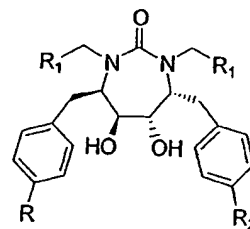
| Structure, R                                                     | Enzymatic assay        |                                |                                        | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|------------------------------------------------------------------|------------------------|--------------------------------|----------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|                                                                  | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub> /<br>nM | 84V9<br>0M<br>IC <sub>50</sub> /<br>nM | WT                                            | 84V9<br>0M | 30N<br>82188<br>D | 48V5<br>4V82<br>A | 48V5<br>4V82<br>S | 48V8<br>2A90<br>M | 46150<br>V |
| CH <sub>3</sub><br>(DMP-851)                                     | 0.033                  | 3.8                            | 9.4                                    | 54                                            | 918        | 69                | 33                | 30                | 22                | 17         |
| OH                                                               | 0.65 <sup>a</sup>      | 6.1                            | 77                                     | 356                                           | 2791       | 669               | 294               | ND                | ND                | 683        |
| OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                 | 1.230 <sub>a</sub>     | 23                             | 157                                    | 356                                           | >600<br>0  | 145               | 175               | ND                | ND                | 138        |
| OCH <sub>2</sub> PO <sub>3</sub> H <sub>2</sub>                  | 0.809                  | 59                             | 137                                    | 1074                                          | >600<br>0  | ND                | ND                | ND                | ND                | ND         |
| O-mono-Lac-Et                                                    | >2.0                   | 93                             | 553                                    | >600<br>0                                     | >600<br>0  | ND                | ND                | ND                | ND                | ND         |
| O-mono-Lac-Bu                                                    | >2.0                   | 25                             | 249                                    | >600<br>0                                     | >600<br>0  | ND                | ND                | ND                | ND                | ND         |
| CH <sub>2</sub> OH                                               | 0.017                  | 2.8                            | 31                                     | 253                                           | 1106       | 486               | 413               | ND                | ND                | 524        |
| CH <sub>2</sub> OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub> | 2.8                    | 13                             | 123                                    | 119                                           | 3295       | 267               | 430               | ND                | ND                | 789        |
| CH <sub>2</sub> OCH <sub>2</sub> PO <sub>3</sub> H <sub>2</sub>  |                        | 42                             | 205                                    | 1757                                          | >424<br>3  | ND                | ND                | ND                | ND                | ND         |



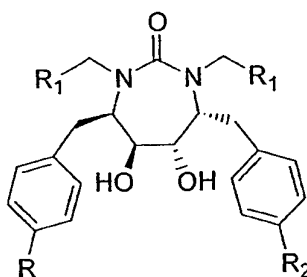
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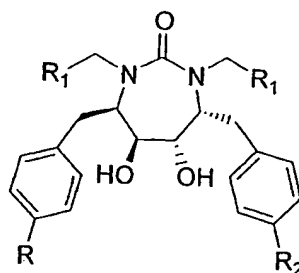


| R   | R1      | R2                               | Enzymatic assay        |                                |                                        | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|-----|---------|----------------------------------|------------------------|--------------------------------|----------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|     |         |                                  | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub> /<br>nM | 84V<br>90M<br>IC <sub>50</sub><br>/ nM | WT                                            | 84V<br>90M | 30N<br>82I8<br>8D | 48V<br>54V<br>82A | 48V<br>54V<br>82S | 48V<br>82A<br>90M | 46I5<br>0V |
| --- | ---     | ---                              | 0.03                   | 3.0                            | 9.1                                    | 165                                           | 819        | 82                | 82                | 73                | 45                | 88         |
| --- | ---     | ---                              | 0.37                   | 5.8                            | 43.3                                   | 193                                           | 2312       | 281               | 705               | ND                | ND                | 772        |
| H   | Ph      | H                                |                        | 34                             | 631                                    | 2492                                          | >600       | 3360              | ND                | ND                | ND                | ND         |
| OH  | Ph      | OH                               |                        | 31                             | 397                                    | 117                                           | 5609       | 756               | 2266              | ND                | ND                | 928        |
| OH  | Ph      | OCH <sub>2</sub> PO <sub>3</sub> |                        | 9                              | 40                                     | 33                                            | 791        | 92                | 807               | 1103              | 1429              | 53         |
| H   | Ph      | OCH <sub>2</sub> PO <sub>3</sub> | 0.65                   | 3.9                            | 48                                     | 107                                           | 2456       | 293               | 1438              | 1899              | 3292              | 589        |
| H   | Indazol | H                                | <0.0                   | 2.5                            | 13                                     | 11                                            | 22         | <8                | 5.5               | 8                 | 4                 | 4.0        |
| OH  | Indazol | OH                               | 0.01                   | 0.6                            | 3.5                                    | >600                                          | 2728       | 7224              | ND                | ND                | ND                | ND         |
| OH  | Indazol | OCH <sub>2</sub> PO <sub>3</sub> | 0.13                   | 1.1                            | 5.5                                    | 1698                                          | 1753       | 1998              | ND                | ND                | ND                | ND         |
| H   | Indazol | OCH <sub>2</sub> PO <sub>3</sub> | 0.02                   | 1.4                            | 6.2                                    | 57                                            | 40         | 68                | 28                | 26                | 32                | 27         |

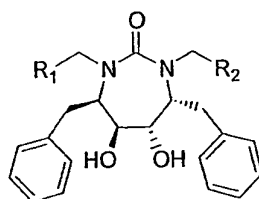


|                  |                              |                                         | Enzymatic assay     |                          |                               | Cell-based assay (MT-4) EC <sub>50</sub> / nM |         |             |             |             |             |         |
|------------------|------------------------------|-----------------------------------------|---------------------|--------------------------|-------------------------------|-----------------------------------------------|---------|-------------|-------------|-------------|-------------|---------|
| R                | R1                           | R2                                      | K <sub>i</sub> (nM) | WT IC <sub>50</sub> / nM | 84V 90M IC <sub>50</sub> / nM | WT                                            | 84V 90M | 30N 82I8 8D | 48V 54V 82A | 48V 54V 82S | 48V 82A 90M | 4615 0V |
| ---              | ---                          | ---                                     | 0.03<br>3           | 3.0                      | 9.1                           | 165                                           | 819     | 82          | 82          | 73          | 45          | 88      |
| OH               | Ph                           | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> |                     | 9                        | 40                            | 33                                            | 791     | 92          | 807         | 1103        | 1429        | 53      |
| H                | Ph                           | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | 0.65<br>6           | 3.9                      | 48                            | 107                                           | 2456    | 293         | 1438        | 1899        | 3292        | 589     |
| OCH <sub>3</sub> | Ph                           | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> |                     |                          |                               |                                               |         |             |             |             |             |         |
| OH               | Ph-pOH                       | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | <0.0<br>1           | 2.6                      | 18                            | 285                                           | 1912    | 211         | 986         | ND          | ND          | 1107    |
| H                | Ph-pOH                       | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | 0.31<br>9           | 2.1                      | 33                            | 65                                            | 272     | 90          | 128         | 198         | 126         | 144     |
| OCH <sub>3</sub> | Ph-pOH                       | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | 0.04<br>5           | 1.8                      | 17                            | 29                                            | 146     | 23          | 67          | 106         | 48          | 68      |
| OH               | Ph-mNH <sub>2</sub><br>/NHEt | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> |                     | 8.7                      | 67                            | 286                                           | 1902    | 562         | 789         | 1781        | 684         | 239     |
| H                | Ph-mNH <sub>2</sub>          | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | 0.12<br>6           | 3.4                      | 39                            | 65                                            | 328     | 16          | 168         | 146         | 74          | 46      |
| OCH <sub>3</sub> | Ph-mNH <sub>2</sub>          | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> | <0.0<br>1           | 3.6                      | 56                            | 63                                            | 535     | 18          | 202         | 117         | 102         | 36      |
| OCH <sub>3</sub> | m-<br>pyridine               | OCH <sub>2</sub> PO<br>3Et <sub>2</sub> |                     |                          |                               | 115                                           | 765     | 106         | 1019        | 970         | 480         | 352     |

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|                  |                     |                                                                          | Enzymatic assay        |                                | Cell-based assay (MT-4) EC <sub>50</sub> / nM |       |            |                   |                   |                   |                   |            |
|------------------|---------------------|--------------------------------------------------------------------------|------------------------|--------------------------------|-----------------------------------------------|-------|------------|-------------------|-------------------|-------------------|-------------------|------------|
| R                | R1                  | R2                                                                       | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub> /<br>nM | 84V<br>90M<br>IC <sub>50</sub><br>/ nM        | WT    | 84V9<br>0M | 30N<br>82I88<br>D | 48V5<br>4V82<br>A | 48V5<br>4V82<br>S | 48V8<br>2A90<br>M | 46I50<br>V |
| ---              | ---                 | ---                                                                      | 0.033                  | 3.0                            | 9.1                                           | 165   | 819        | 82                | 82                | 73                | 45                | 88         |
| H                | Ph-mNH <sub>2</sub> | OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                         | 0.126                  | 3.4                            | 39                                            | 65    | 328        | 16                | 168               | 146               | 74                | 46         |
| OCH <sub>3</sub> | Ph-mNH <sub>2</sub> | OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                         | <0.01                  | 3.6                            | 56                                            | 63    | 535        | 18                | 202               | 117               | 102               | 36         |
| OCH <sub>3</sub> | Ph-mNH <sub>2</sub> | O(CH <sub>2</sub> ) <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>         |                        |                                |                                               |       |            |                   |                   |                   |                   |            |
| OCH <sub>3</sub> | Ph-mNH <sub>2</sub> | OCONH<br>(CH <sub>2</sub> ) <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub> |                        | 11.3                           | 116                                           | 74    | 2265       | 77                | 262               | 214               | 215               | 184        |
| OCH <sub>3</sub> | Ph-mNH <sub>2</sub> | OCONH<br>(CH <sub>2</sub> )PO <sub>3</sub> Et <sub>2</sub>               |                        | 9.9                            | 85                                            | 58    | 2151       | 68                | 223               | 203               | 185               | 104        |
| H                | Ph-pOH              | OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                         | 0.319                  | 2.1                            | 33                                            | 65    | 272        | 90                | 128               | 222               | 146               | 144        |
| OCH <sub>3</sub> | Ph-pOH              | OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                         | 0.045                  | 1.8                            | 17                                            | 30    | 148        | 25                | 70                | 129               | 54                | 90         |
| OCH <sub>3</sub> | Ph-pOH              | OCONH<br>(CH <sub>2</sub> ) <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub> |                        | 6.6                            | 49                                            | 33    | 495        | 31                | 74                | 51                | 55                | 223        |
| ---              | ---                 | ---                                                                      | 0.033                  | 3.0                            | 9.1                                           | 165   | 819        | 82                | 82                | 73                | 45                | 88         |
| H                | Ph                  | OCH <sub>2</sub> PO <sub>3</sub> Et <sub>2</sub>                         | 0.656                  | 3.9                            | 48                                            | 107   | 2456       | 293               | 1438              | 1899              | 3292              | 589        |
| H                | Ph                  | OH                                                                       | 0.330                  | 15                             | 162                                           | 1261  | >6000      | 2952              | >6000             |                   |                   |            |
| H                | Ph                  | OCH <sub>2</sub> PO <sub>3</sub> Bn <sub>2</sub>                         | 0.125                  | 7.4                            | 158                                           | 1769  | >6000      | 3135              | >6000             |                   |                   |            |
| H                | Ph                  | OCH <sub>2</sub> PO <sub>3</sub> H <sub>2</sub>                          | 0.386                  | 9.7                            | 210                                           | >6000 | >6000      | ND                | ND                |                   |                   |            |
| H                | Ph                  | Mono-lac-Et                                                              | 0.120                  | 6.6                            | 56                                            | 1726  | >6000      | 2793              | >6000             |                   |                   |            |
| H                | Ph                  | Mono-Ala-Et                                                              |                        | 5                              | 50                                            | 310   | 2943       | 238               | 2851              | 1948              | 2450              | 1250       |



| R1     | R2 | Enzymatic assay        |                                |                                            | Cell-based assay (MT-4) EC <sub>50</sub> / nM |            |                   |                   |                   |                   |            |
|--------|----|------------------------|--------------------------------|--------------------------------------------|-----------------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------|
|        |    | K <sub>i</sub><br>(nM) | WT<br>IC <sub>50</sub><br>/ nM | 84V<br>90<br>M<br>IC <sub>50</sub><br>/ nM | WT                                            | 84V9<br>0M | 30N<br>82I88<br>D | 48V5<br>4V82<br>A | 48V5<br>4V82<br>S | 48V8<br>2A90<br>M | 46L50<br>V |
| Phenyl |    | 0.03                   | 3.0                            | 9.1                                        | 165                                           | 819        | 82                | 82                | 73                | 45                | 88         |
| Phenyl |    | 0.42                   | 6.6                            | 85                                         | 1226                                          | >600       | 869               | 774               | ND                | ND                | 937        |
| Phenyl |    | 0.37                   | 5.8                            | 43.3                                       | 193                                           | 2312       | 281               | 705               | ND                | ND                | 772        |
| Phenyl |    |                        | 109                            | >25                                        | >600                                          | ND         | ND                | ND                | ND                | ND                | ND         |
| Phenyl |    |                        |                                |                                            |                                               |            |                   |                   |                   |                   |            |
| Phenyl |    |                        |                                |                                            |                                               |            |                   |                   |                   |                   |            |
| Phenyl |    |                        |                                |                                            |                                               |            |                   |                   |                   |                   |            |
|        |    | 1.43                   | 302                            | 114                                        | >600                                          | >600       | ND                | ND                | ND                | ND                | ND         |
|        |    | >5                     | >25                            | ND                                         | 5949                                          | ND         | ND                | ND                | ND                | ND                | ND         |
|        |    | >5                     | 130                            | 348                                        | 2006                                          | 3121       | ND                | ND                | ND                | ND                | ND         |

All publications and patent applications cited herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although certain embodiments have been described in detail above, those having ordinary skill in the art will clearly understand that many modifications are possible in the embodiments without departing from the teachings thereof. All such modifications are intended to be encompassed within the claims of the invention.



Example: Preliminary Study: Plasma and PBMC Exposure Following Intravenous and Oral Administration of Candidate to Beagle Dogs

The pharmacokinetics of a phosphonate prodrug GS77366 (P1-monoLac-iPr, structure shown below), its active metabolite (metabolite X, or GS77568), and GS8373 were studied in dogs following intravenous and oral administration of the candidate.

Dose Administration and Sample Collection. The in-life phase of this study was conducted in accordance with the USDA Animal Welfare Act and the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and followed the standards for animal husbandry and care found in the Guide for the Care and Use of Laboratory Animals, 7<sup>th</sup> Edition, Revised 1996. All animal housing and study procedures involving live animals were carried out at a facility which had been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care - International (AAALAC).

Each animal in a group of 4 female beagle dogs was given a bolus dose of GS77366 (P1-monoLac-iPr) intravenously at 1 mg/kg in a formulation containing 40% PEG 300, 20% propylene glycol and 40% of 5% dextrose. Another group of 4 female beagle dogs was dosed with GS77366 via oral gavage at 20 mg/kg in a formulation containing 60% Vitamin-E TPGS, 30% PEG 400 and 10% propylene glycol.

Blood samples were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr and 24 hr post-dose. Plasma (0.5 to 1 mL) was prepared from each sample and kept at -70°C until analysis. Blood samples (8 mL) were also collected from each dog at 2, 8 and 24 hr post dose in Becton-Dickinson CPT vacutainer tubes. PBMCs were isolated from the blood by centrifugation for 15 minutes at 1500 to 1800 G. After centrifugation, the fraction containing PBMCs was transferred to a 15 mL conical centrifuge tube and the PBMCs were washed twice with phosphate buffered saline (PBS) without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . The final wash of the cell pellet was kept at -70°C until analysis.

Measurement of the candidate, metabolite X and GS8373 in plasma and PBMCs. For plasma sample analysis, the samples were processed by a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10  $\mu\text{M}$ , from J.T. Baker) were conditioned with 200  $\mu\text{L}$  of methanol followed by 200  $\mu\text{L}$  of water. An aliquot of 200  $\mu\text{L}$  of plasma sample was applied to each cartridge, followed by two washing

steps each with 200  $\mu$ L of deionized water. The compounds were eluted from the cartridges with a two-step process each with 125  $\mu$ L of methanol. Each well was added 50  $\mu$ L of water and mixed. An aliquot of 25  $\mu$ L of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5  $\mu$ m) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, pH 3.0. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, pH 4.6. The chromatography was carried out at a flow rate of 250  $\mu$ L/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure GS77366, GS8373 and Metabolite X with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for GS77366, GS8373 and GS77568 (Metabolite X) in plasma.

For PBMC sample analysis, phosphate buffered saline (PBS) was added to each PBMC pellet to bring the total sample volume to 500  $\mu$ L in each sample. An aliquot of 150  $\mu$ L from each PBMC sample was mixed with an equal volume of methanol, followed by the addition of 700  $\mu$ L of 1% formic acid in water. The resulting mixture was applied to a Speedisk C18 solid phase extraction cartridge (1 mL, 20 mg, 10  $\mu$ m, from J.T. Baker) which had been conditioned as described above. The compounds were eluted with methanol after washing the cartridge 3 times with 10% methanol. The solvent was evaporated under a stream of N<sub>2</sub>, and the sample was reconstituted in 150  $\mu$ L of 30% methanol. An aliquot of 75  $\mu$ L of the solution was injected for LC/MS/MS analysis. The limit of quantitation was 0.1 ng/mL in the PBMC suspension.

Pharmacokinetic Calculations. The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were calculated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

Plasma and PBMC Concentration-time Profiles. The following shows the concentration-time profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous dosing of GS77366 at 1 mg/kg in dogs. The data demonstrate that the prodrug can effectively deliver the active components (metabolite X and GS8373) into cells that are primarily responsible for HIV replication, and that the active components in these cells had much longer half-life than in plasma.

Pharmacokinetic profiles of GS77366, GS77568 and GS8373 in plasma and PBMCs following intravenous administration of GS77366 at 1 mg/kg in dogs are shown in fig. 6.

The pharmacokinetic properties of GS77568 in PBMCs following oral administration of GS77366 in dogs are compared with that of nelfinavir and amprenavir, two marketed HIV protease inhibitors. These data show that the active component (GS77568) from the phosphonate prodrug had sustained levels in PBMCs compared to nelfinavir and amprenavir.

Concentration-time profiles of GS77568, nelfinavir and amprenavir in PBMCs following oral administration of GS77366 (20 mg/kg), nelfinavir (17.5 mg/kg) and amprenavir (20 mg/kg) in dogs are shown in fig. 7.

**Table 1a. Comparison of GS77568 with nelfinavir and amprenavir in PBMCs following oral administration in beagle dogs.**

| Compound   | Dose                | $t_{1/2}$ (hr) | AUC <sub>(2-24 hr)</sub> |
|------------|---------------------|----------------|--------------------------|
| Nelfinavir | 17.5 mg/kg          | 3.0 hr         | 33,000 nM·hr             |
| Amprenavir | 20 mg/kg            | 1.7 hr         | 102,000 nM·hr            |
| GS77568    | 20 mg/kg of GS77366 | > 20 hr        | 42,200 nM·hr             |

#### Intracellular Metabolism/In Vitro Stability

##### 1. Uptake and Persistence in MT2 cells, quiescent and stimulated PBMC

The protease inhibitor (PI) phosphonate prodrugs undergo rapid cell uptake and metabolism to produce acid metabolites including the parent phosphonic acid. Due to the presence of charges, the acid metabolites are significantly more persistent in the cells than non-charged PI's. In order to estimate the relative intracellular levels of the different PI prodrugs, three

phosphonate, monoamidate phenoxy phosphonate and monolactate phenoxy phosphonate (Figure 1) were incubated at 10  $\mu$ M for 1 hr with MT-2 cells, stimulated and quiescent peripheral blood mononuclear cells (PBMC) (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 24 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. Typically, the cell lysates were centrifuged and 100  $\mu$ L of the supernatant were mixed with 200  $\mu$ L of 7.5  $\mu$ M amprenavir (Internal Standard) in 80% acetonitrile/20% water and injected into an HPLC system (70  $\mu$ L).

HPLC Conditions:

Analytical Column: Prodigy ODS-3, 75 x 4.6, 3 $\mu$  + C18 guard at 40°C

Gradient:

Mobile Phase A: 20 mM ammonium acetate in 10% ACN/90% H<sub>2</sub>O

Mobile Phase B: 20 mM ammonium acetate in 70% ACN/30% H<sub>2</sub>O

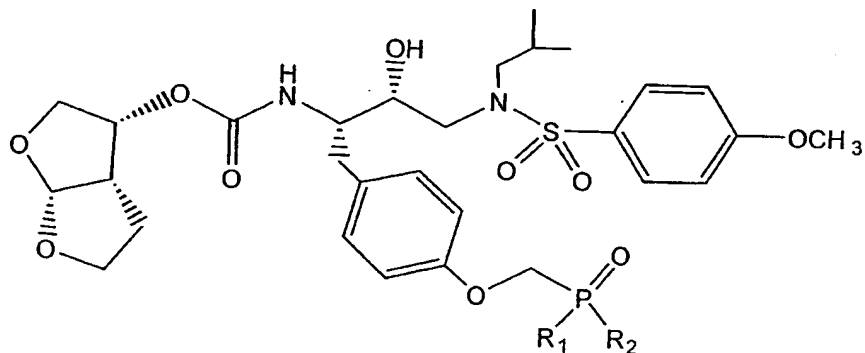
30-100%B in 4 min, 100%B for 2 min, 30%B for 2 min at 2.5 mL/min.

Run Time: 8 min

UV Detection @ 245 nm

Concentrations of Intracellular metabolites were calculated based on cell volume 0.2  $\mu$ L/mln cells for PBMC and 0.338  $\mu$ L / mln (0.676  $\mu$ L / mL) for MT-2 cells.

**Chemical Structures of Selected Protease Inhibitor Phosphonate Prodrugs and Intracellular Metabolites.**



| GS No. | R1                          | R2                                          | EC <sub>50</sub> (nM) |
|--------|-----------------------------|---------------------------------------------|-----------------------|
| 8373   | OH                          | OH                                          | 4,800±1,800           |
| 16503  | HNCH(CH <sub>3</sub> )COOBu | HNCH(CH <sub>3</sub> )COOBu                 | 6.0±1.4               |
| 16571  | OPh                         | HNCH(CH <sub>3</sub> )COOEt                 | 15±5                  |
| 17394  | OPh                         | OCH(CH <sub>3</sub> )COOEt                  | 20±7                  |
| 16576  | OPh                         | HNCH(CH <sub>2</sub> CH <sub>3</sub> )COOEt | 12.6±4.8              |
| Met X  | OH                          | HNCH(CH <sub>3</sub> )COOH                  | >10,000               |
| Met LX | OH                          | OCH(CH <sub>3</sub> )COOEt                  | 1750±354              |

The foregoing data demonstrates that there was a significant uptake and conversion of all 3 compounds in all cell types. The uptake in the quiescent PBMC was 2-3-fold greater than in the stimulated cells. GS-16503 and GS-16571 were metabolized to Metabolite X and GS-8373. GS-17394 metabolized to the Metabolite LX. Apparent intracellular half-lives were similar for all metabolites in all cell types (7-12 hr).

Persistence of Total Acid Metabolites of Protease Inhibitor Prodrugs in Stimulated (A), Quiescent PBMC (B) and MT-2 Cells (C) (1 hr, 10 uM Pulse, 24 hr Chase) is shown in figures 8 to 10.

## 2. Uptake and Persistence in Stimulated and Quiescent T-cells

Since HIV mainly targets T-lymphocytes, it is important to establish the uptake, metabolism and persistence of the metabolites in the human T-cells. In order to estimate the relative intracellular levels of the different PI prodrugs, GS-16503, 16571 and 17394 were incubated at 10  $\mu$ M for 1 hr with quiescent and stimulated T-cells (pulse phase). The prodrugs were compared with a non-prodrug PI, nelfinavir. After incubation, the cells were washed, resuspended in the cell culture media and incubated for 4 hr (chase phase). At specific time points, the cells were washed, lysed and the lysates were analyzed by HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC.

Table 1b demonstrates the levels of total acid metabolites and corresponding prodrugs in T-cells following pulse/chase and continuous incubation. There was significant cell uptake/metabolism in T-lymphocytes. There was no apparent difference in uptake between stimulated and quiescent T-lymphocytes. There was significantly higher uptake of phosphonate PI's than nelfinavir. GS17394 demonstrates higher intracellular levels than GS16571 and GS16503. The degree of conversion to acid metabolites varied between different prodrugs. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equal mixture of the mono-phosphonic acid metabolite and GS-8373 except for GS-17394, where Metabolite LX was stable, with no GS-8373 formed.

**Table 1b. Intracellular Levels of Metabolites and Intact Prodrug Following Continuous and 1 hr Pulse/4 hr Chase Incubation (10  $\mu$ M/0.7 mln cells/1 mL) of 10  $\mu$ M PI Prodrugs and Nelfinavir with Quiescent and Stimulated T-cells**

| Compound   | Time (h) | Continuous Incubation |                    |                     |                    | 1 hr Pulse /4 hr Chase |                    |                     |                    |
|------------|----------|-----------------------|--------------------|---------------------|--------------------|------------------------|--------------------|---------------------|--------------------|
|            |          | Quiescent T-cells     |                    | Stimulated T-cells  |                    | Quiescent T-cells      |                    | Stimulated T-cells  |                    |
|            |          | Acid Met ( $\mu$ M)   | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M) | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M)    | Prodrug ( $\mu$ M) | Acid Met ( $\mu$ M) | Prodrug ( $\mu$ M) |
| 16503      | 0        | 1180                  | 42                 | 2278                | 0                  | 2989                   | 40                 | 1323                | 139                |
|            | 2        | 3170                  | 88                 | 1083                | 116                | 1867                   | 4                  | 1137                | 31                 |
|            | 4        | 5262                  | 0                  | 3198                | 31                 | 1054                   | 119                | 1008                | 0                  |
| 16571      | 0        | 388                   | 1392               | 187                 | 1417               | 1042                   | 181                | 858                 | 218                |
|            | 2        | 947                   | 841                | 1895                | 807                | 1170                   | 82                 | 1006                | 35                 |
|            | 4        | 3518                  | 464                | 6147                | 474                | 1176                   | 37                 | 616                 | 25                 |
| 17394      | 0        | 948                   | 1155               | 186                 | 1194               | 4480                   | 14                 | 2818                | 10                 |
|            | 2        | 7231                  | 413                | 3748                | 471                | 2898                   | 33                 | 1083                | 51                 |
|            | 4        | 10153                 | 167                | 3867                | 228                | 1548                   | 39                 | 943                 | 104                |
| Nelfinavir | 0        |                       | 101                |                     | 86                 |                        | 886                |                     | 1239               |
|            | 2        |                       | 856                |                     | 846                |                        | 725                |                     | 770                |
|            | 4        |                       | 992                |                     | 1526               |                        | 171                |                     | 544                |

### 3. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1 $\mu$ M.

To determine if the cell uptake/metabolism is concentration dependent, selected PI's were incubated with the 1 mL of MT-2 cell suspension (2.74 mln cells/mL) for 1 hr at 37°C at 3 different concentrations: 10, 5 and 1  $\mu$ M. Following incubation, cells were washed twice with the cell culture medium, lysed and assayed using HPLC with UV detection. The sample preparation and analysis were similar to the ones described for MT-2 cells, quiescent and stimulated PBMC. Intracellular concentrations were calculated based on cell count, a published single cell volume of 0.338 pl for MT-2 cells, and concentrations of analytes in cell lysates. Data are shown in Table 2a.

Uptake of all three selected PI's in MT-2 cells appears to be concentration-independent in the 1-10  $\mu$ M range. Metabolism (conversion to acid metabolites) appeared to be concentration-dependent for GS-16503 and GS-16577 (3-fold increase at 1  $\mu$ M vs. 10  $\mu$ M) but independent for GS-17394 (monolactate). Conversion from a respective metabolite X to GS-8373 was concentration-independent for both GS-16503 and GS-16577 (no conversion was observed for metabolite LX of GS-17394).

**Table 2a. Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in MT-2 Cells at 10, 5 and 1  $\mu$ M.**

| Compound | Extracellular Concentration, $\mu$ M | Cell-Associated Prodrug and Metabolites Concentration, $\mu$ M |        |         |       | % Conversion to acid metabolites |
|----------|--------------------------------------|----------------------------------------------------------------|--------|---------|-------|----------------------------------|
|          |                                      | Metabolite X                                                   | GS8373 | Prodrug | Total |                                  |
| GS-17394 | 10                                   | 1358                                                           | 0      | 635     | 1993  | 68                               |
|          | 5                                    | 916                                                            | 0      | 449     | 1365  | 67                               |
|          | 1                                    | 196                                                            | 0      | 63      | 260   | 76                               |
| GS-16576 | 10                                   | 478                                                            | 238    | 2519    | 3235  | 22                               |
|          | 5                                    | 250                                                            | 148    | 621     | 1043  | 40                               |
|          | 1                                    | 65                                                             | 36     | 61      | 168   | 64                               |
| GS-16503 | 10                                   | 120                                                            | 86     | 1506    | 1712  | 12                               |
|          | 5                                    | 58                                                             | 60     | 579     | 697   | 17                               |
|          | 1                                    | 12                                                             | 18     | 74      | 104   | 29                               |

\* For GS16576, Metabolite X is mono-aminobutyric acid



#### 4. PBMC Uptake and Metabolism of Selected PI Candidates Following 1-hr Incubation in Human Whole Blood at 10 $\mu$ M.

In order to estimate the relative intracellular levels of the different PI prodrugs candidates under conditions simulating the in vivo environment, compounds representative of three classes of phosphonate PI prodrugs – bisamidate phosphonate (GS-16503), monoamidate phenoxy phosphonate (GS-16571) and monolactate phenoxy phosphonate (GS-17394) (Figure 1) were incubated at 10  $\mu$ M for 1 hr with intact human whole blood at 37°C. After incubation, PBMC were isolated, then lysed and the lysates were analyzed by HPLC with UV detection.

The results of analysis are shown in Table 3. There was significant cell uptake/metabolism following incubation in whole blood. There was no apparent difference in uptake between GS-16503 and GS-16571. GS-17394 demonstrated significantly higher intracellular levels than GS-16571 and GS-16503.

The degree of conversion to acid metabolites varies between different prodrugs after 1 hr incubation. GS-17394 demonstrated the highest degree of conversion, followed by GS-16503 and GS-16571. The metabolites, generally, were an equimolar mixture of the mono-phosphonic acid metabolite and GS-8373 (parent acid) except for GS-17394, where Metabolite LX was stable with no GS-8373 formed.

**Table 3a. PBMC Uptake and Metabolism of Selected PI Prodrugs Following 1-hr Incubation in Human Whole Blood at 10  $\mu$ M (Mean  $\pm$  SD, N=3).**

| GS#   | Intracellular Prodrug and Metabolites Concentration, $\mu$ M |                  |                | Major Intracellular Metabolites |
|-------|--------------------------------------------------------------|------------------|----------------|---------------------------------|
|       | Acid Metabolite                                              | Prodrug, $\mu$ M | Total, $\mu$ M |                                 |
| 16503 | 279 $\pm$ 47                                                 | 61 $\pm$ 40      | 340 $\pm$ 35   | X, GS-8373                      |
| 16571 | 319 $\pm$ 112                                                | 137 $\pm$ 62     | 432 $\pm$ 208  | X, GS-8373                      |
| 17394 | 629 $\pm$ 303                                                | 69 $\pm$ 85      | 698 $\pm$ 301  | LX                              |

\* PBMC Intracellular Volume = 0.2  $\mu$ L/mln

#### 5. Distribution of PI Prodrug Candidates in PBMC

In order to compare distribution and persistence of PI phosphonate prodrugs with those of non-prodrug PI's, GS-16503, GS-17394 and nelfinavir, were incubated at 10  $\mu$ M for 1 hr with PBMC (pulse phase). After incubation, the cells were washed, resuspended in the cell culture media and incubated for 20 more hr (chase phase). At specific time points, the cells were washed and lysed. The cell cytosol was separated from membranes by centrifugation at 9000 x g. Both cytosol and membranes were extracted with acetonitrile and analyzed by HPLC with UV detection.

Table 4a and the accompanying bar graphs below show the levels of total acid metabolites and corresponding prodrugs in the cytosol and membranes before and after the 22 hr chase. Both prodrugs exhibited complete conversion to the acid metabolites (GS-8373 and X for GS-16503 and LX for GS-17394, respectively). The levels of the acid metabolites of the PI phosphonate prodrugs in the cytosol fraction were 2-3-fold greater than those in the membrane fraction after the 1 hr pulse and 10-fold greater after the 22 hr chase. Nelfinavir was present only in the membrane fractions. The uptake of GS-17394 was about 3-fold greater than that of GS-16503 and 30-fold greater than nelfinavir.

The metabolites were an equimolar mixture of metabolite X and GS-8373 (parent acid) for GS-16503 and only metabolite LX for GS-17394.

**Table 4a. Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following Continuous and 1 hr Pulse/22 hr Chase Incubation of 10  $\mu$ M PI Prodrugs and Nelfinavir with Quiescent PBMC.**

| GS#        | Cell Type | Fraction | Cell-Associated PI, pmol/mln cells |         |                         |         |
|------------|-----------|----------|------------------------------------|---------|-------------------------|---------|
|            |           |          | 1 hr Pulse/ 0 hr Chase             |         | 1 hr Pulse/ 22 hr Chase |         |
|            |           |          | Acid Metabolites                   | Prodrug | Acid Metabolites        | Prodrug |
| GS-16503   | PBMC      | Membrane | 228                                | 0       | 9                       | 0       |
| GS-16503   | PBMC      | Cytosol  | 390                                | 0       | 130                     | 0       |
| GS-17394   | PBMC      | Membrane | 335                                | 0       | 26                      | 0       |
| GS-17394   | PBMC      | Cytosol  | 894                                | 0       | 249                     | 0       |
| Nelfinavir | PBMC      | Membrane |                                    | 42      |                         | 25      |
| Nelfinavir | PBMC      | Cytosol  |                                    | 0       |                         | 0       |

Uptake and Cell Distribution of Metabolites and Intact Prodrugs Following 1 hr Pulse/22 hr Chase Incubation of 10  $\mu$ M PI Prodrugs and Nelfinavir with Quiescent PBMC is shown in fig. 11 and fig. 12.

#### 6. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrug Candidates

The in vitro metabolism and stability of the PI phosphonate prodrugs were determined in PBMC extract, dog plasma and human serum. Biological samples listed below (120  $\mu$ L) were transferred into an 8-tube strip placed in the aluminum 37°C heating block/holder and incubated at 37°C for 5 min. Aliquots (2.5  $\mu$ L) of solution containing 1 mM of test compounds in DMSO, were transferred to a clean 8-tube strip, placed in the aluminum 37°C heating block/holder. 60  $\mu$ L aliquots of 80% acetonitrile/20% water containing 7.5  $\mu$ M of amprenavir as an internal standard for HPLC analysis were placed into five 8-tube strips and kept on ice/refrigerated prior to use. An enzymatic reaction was started by adding 120  $\mu$ L aliquots of a biological sample to the strip with the test compounds using a multichannel pipet. The strip was immediately vortex-mixed and the reaction mixture (20  $\mu$ L) was sampled and transferred to the Internal Standard/ACN strip. The sample was considered the time-zero sample (actual time was 1-2 min). Then, at specific time points, the reaction mixture (20  $\mu$ L) was sampled and transferred to the corresponding IS/ACN strip.

sampling times were 6, 20, 60 and 120 min. When all time points were sampled, an 80  $\mu$ L aliquot of water was added to each tube and strips were centrifuged for 30 min at 3000xG.

The supernatants were analyzed with HPLC under the following conditions:

Column: Inertsil ODS-3, 75 x 4.6 mm, 3  $\mu$ m at 40°C.

Mobile Phase A: 20 mM ammonium acetate in 10%ACN/90%water

Mobile Phase B 20 mM ammonium acetate in 70%ACN/30%water

Gradient: 20% B to 100% B in 4 min, 2 min 100% B, 2 min 20% B

Flow Rate: 2 mL/min

Detection: UV at 243 nm

Run Time: 8 min

The biological samples evaluated were as follows:

**PBMC cell extract** was prepared from fresh cells using a modified published procedure (A. Pompon, I. Lefebvre, J.-L. Imbach, S. Kahn, and D. Farquhar, Antiviral Chemistry & Chemotherapy, 5, 91 - 98 (1994)). Briefly, the extract was prepared as following: The cells were separated from their culture medium by centrifugation (1000 g, 15 min, ambient temperature). The residue (about 100  $\mu$ L,  $3.5 \times 10^8$  cells) was resuspended in 4 mL of a buffer (0.010 M HEPES, pH 7.4, 50 mM potassium chloride, 5 mM magnesium chloride and 5 mM dl-dithiothreitol) and sonicated. The lysate was centrifuged (9000 g, 10 min, 4°C) to remove membranes. The upper layer (0.5 mg protein/mL) was stored at -70°C. The reaction mixture contained the cell extract at about 0.5 mg protein/mL.

**Human serum** (pooled normal human serum from George King Biomedical Systems, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

**Dog Plasma** (pooled normal dog plasma (EDTA) from Pel Freez, Inc.). Protein concentration in the reaction mixture was about 60 mg protein/mL.

Table 5a. PBMC Extract/Dog Plasma/Human Serum Stability of Selected PI Prodrugs

| GS#   | PBMC<br>Extract <sup>1</sup><br>T <sub>1/2</sub> , min | Dog<br>Plasma<br>T <sub>1/2</sub> , min | Human<br>Serum<br>T <sub>1/2</sub> , min | HIV EC <sub>50</sub><br>(nM) |
|-------|--------------------------------------------------------|-----------------------------------------|------------------------------------------|------------------------------|
| 16503 | 2                                                      | 368                                     | >>400                                    | 6.0 ± 1.4                    |
| 16571 | 49                                                     | 126                                     | 110                                      | 15 ± 5                       |
| 17394 | 15                                                     | 144                                     | 49                                       | 20 ± 7                       |

Example: Pharmacokinetics in Plasma and PBMC Following Intravenous or Oral Administration of Candidate compounds to Beagle Dogs; Method for Determining Intracellular Residence Time

The pharmacokinetics of several candidate compounds and their active metabolites were studied in beagle dogs following intravenous or oral administration of each candidate compound.

**Dose Administration and Sample Collection.** Each dosing group consisted of 3 male beagle dogs that were fasted overnight before dosing. For intravenous administration, each dog was dosed with the candidate compound at 1 mg/kg via the cephalic vein as a slow bolus injection over approximately 1 minute. Blood samples (1-2 mL) were collected from the jugular vein pre-dose, and at 2 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 24 hr post-dose into tubes containing EDTA as the anticoagulant. For oral administration, each dog was dosed with the candidate compound at 4 mg/kg through oral gavage. Blood samples (1-2 mL) were collected pre-dose, and at 5 min, 15 min, 30 min, 1 hr, 2 hr, 4 hr, 8 hr and 12 hr post-dose into tubes containing EDTA as the anticoagulant. The blood samples were stored on ice and plasma samples were obtained by centrifugation within 1 hour after blood collection. The plasma samples were stored at approximately -70°C until analysis for the concentrations of the candidate compound and its metabolites in plasma.

Another set of blood samples was also collected from the jugular vein for evaluation of the concentrations of candidate compound and its metabolites in peripheral blood mononuclear cells (PBMCs). Approximately 8 mL of blood was collected either at 1 hr, 4 hr, 8 hr and 24 hr post-dose or at 2 hr, 8 hr and 24 hr post-dose from the jugular vein into tubes containing EDTA as the anticoagulant. An equal volume of sterile phosphate buffered saline (PBS) was

(Amersham Biosciences) in a 50 mL conical tube. The tube was centrifuged at approximately 500 g for 30 min at room temperature. The upper layer containing plasma was drawn off and discarded. The layer below the plasma layer is enriched with PBMCs. This layer was collected with a clean pipette and transferred to a 15 mL conical tube. The PBMC suspension was centrifuged at approximately 500 g for 10 min at room temperature. The resulting pellet was resuspended in 5 mL of sterile PBS and then centrifuged at approximately 500 g for 10 min at room temperature. The supernatant was removed and 0.5 mL of acetonitrile was added to the pellet. The tube was vortexed, sealed and stored at -70°C until analysis for concentrations of the candidate compound and its metabolites.

**Determination of the concentrations of the candidate compound and its metabolites in plasma.** The plasma concentrations of the candidate compound and its metabolites were determined by an LC/MS/MS assay. The plasma samples were processed with a solid phase extraction (SPE) procedure outlined below. Speedisk C18 solid phase extraction cartridges (1 mL, 20 mg, 10 µm, from J.T. Baker) in a 96-well plate were conditioned with 200 µL of methanol followed by 200 µL of water. An aliquot of 200 µL of plasma sample was applied to each cartridge, followed by two washing steps each with 200 µL of deionized water. The analytes were eluted from the cartridges by a two-step process each with 125 µL of methanol. Each well was added 50 µL of water and mixed to reduce the organic strength. An aliquot of 25 µL of the mixture was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography (LC) was HyPURITY® C18 (50 x 2.1 mm, 3.5 µm) from Thermo-Hypersil. Mobile phase A contained 10% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. Mobile phase B contained 90% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. The chromatography was carried out at a flow rate of 250 µL/min under an isocratic condition of 40% mobile phase A and 60% mobile phase B. Selected reaction monitoring (SRM) were used to measure the candidate compound and its metabolites simultaneously with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 1 nM for the candidate compound and its metabolites in plasma.

**Determination of the concentrations of the candidate compound and its metabolites in PBMCs.**

The concentrations of the candidate compound and its metabolites in PBMCs were determined by an LC/MS/MS assay. The PBMC samples were filtered through a Captiva™ filtration plate with 0.2 µm pore size. An aliquot of 250 µL of the filtrate was evaporated under a stream of nitrogen. The samples were reconstituted in 75 µL of 20% acetonitrile in 0.1% formic acid. An aliquot of 25 µL of the solution was injected onto a ThermoFinnigan TSQ Quantum LC/MS/MS system.

The column used in liquid chromatography was HyPURITY® C18 (50 x 2.1 mm, 3.5 µm) from Thermo-Hypersil. Mobile phase A (MPA) contained 10% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. Mobile phase B (MPB) contained 90% acetonitrile in 10 mM ammonium formate, 0.1% formic acid. The chromatography was carried out at a flow rate of 300 µL/min with a gradient elution program: 5% MPB from 0 to 1.5 min; 5-95% MPB from 1.5 to 1.6 min; 95% MPB from 1.6 to 3.5 min; 95-5% MPB from 3.5 to 3.6 min; 5% MPB till the end of the program (6 min). The first 2 min of the LC flow was diverted to waste to alleviate salt buildup in the probe of the mass spectrometer. Selected reaction monitoring was used to measure the candidate compound and its metabolites simultaneously with the positive ionization mode on the electrospray probe. The limit of quantitation (LOQ) was 0.1 nM for the candidate compound and its metabolites in PBMC suspension.

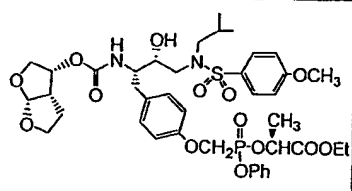
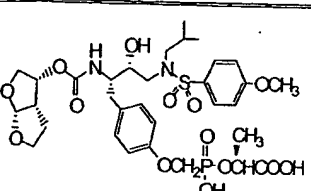
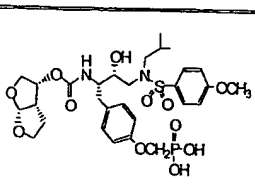
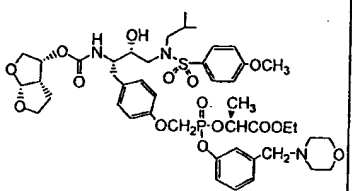
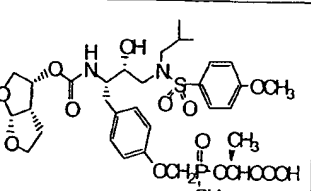
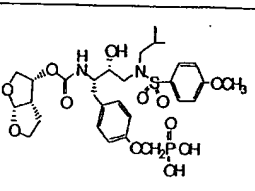
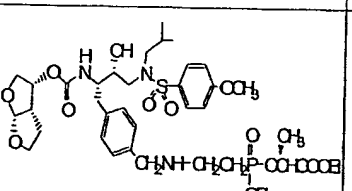
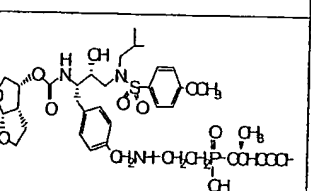
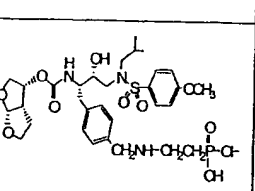
**Pharmacokinetic Calculations.** The pharmacokinetic parameters were calculated using WinNonlin. Noncompartmental analysis was used for all pharmacokinetic calculation. The intracellular concentrations in PBMCs were extrapolated from the measured concentrations in PBMC suspension on the basis of a reported volume of 0.2 picoliter/cell (B.L. Robins, R.V. Srinivas, C.Kim, N.Bischofberger, and A.Fridland, (1998) Antimicrob. Agents Chemother. 42, 612).

**Pharmacokinetic Profiles in Plasma and PBMC.** Shown below are the concentration-time profiles of three phosphonate candidate compounds (GS-1, GS-2 and GS-3) and their metabolites in plasma and PBMCs following intravenous administration of each candidate compound at 1 mg/kg in dogs. The last profile shows the concentration-time profiles of GS-3 and its metabolites in plasma and PBMC following oral administration of GS-3 at 4 mg/kg in dogs. The chemical structures of the candidate compounds and their metabolites are shown in Table 1aa. The data demonstrate that the candidate compounds and their metabolites are present in both plasma and PBMCs following intravenous and oral administration.

active components (metabolite X and diacid) into cells that are primarily associated with HIV activity, and that the half-lives of the active components in these cells are much longer than in plasma.



**Table 1aa.** Chemical Structures of Candidate compounds and Their Metabolites.

|      | Candidate compound                                                                 | Metabolites                                                                         |                                                                                      |
|------|------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
|      |                                                                                    | Metabolite X (MX)                                                                   | Diacid                                                                               |
| GS-1 |   |   |   |
| GS-2 |   |   |   |
| GS-3 |  |  |  |

Pharmacokinetic profiles of GS-1 and its metabolites in plasma and PBMCs following intravenous administration of GS-1 at 1 mg/kg in dogs are shown in figure 13..

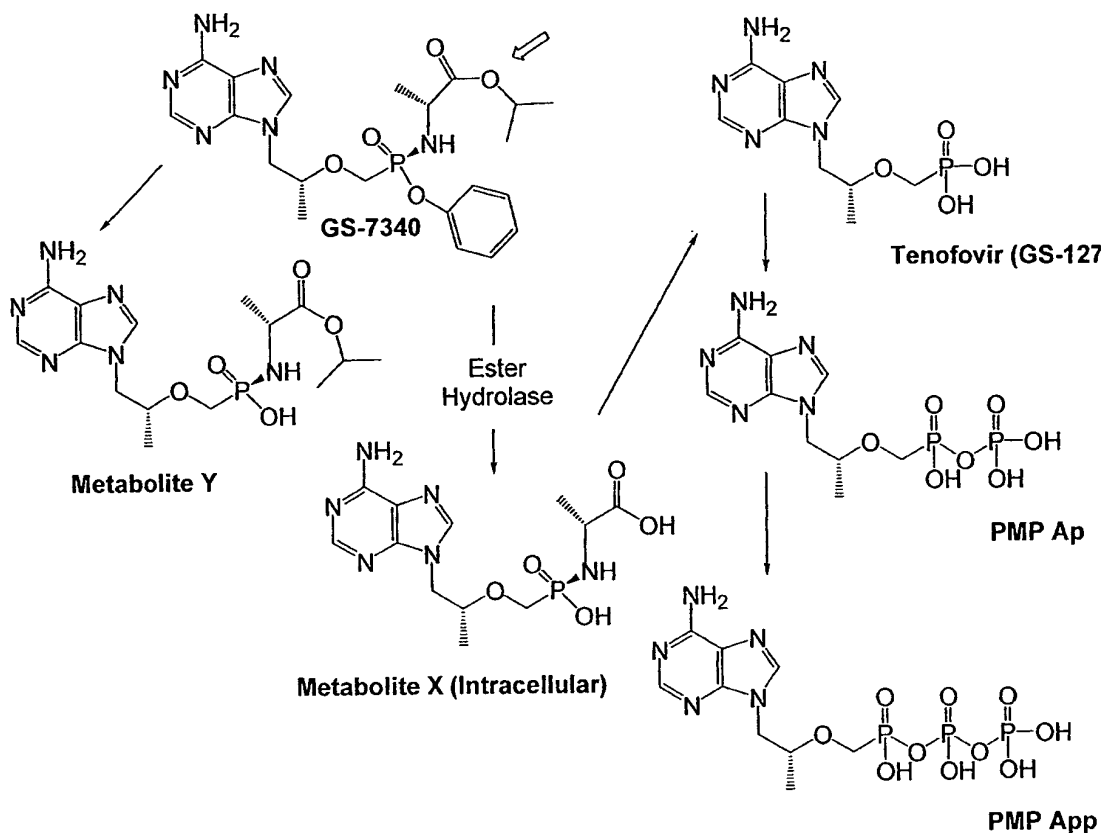
Pharmacokinetic profiles of GS-2 and its metabolites in plasma and PBMCs following intravenous administration of GS-2 at 1 mg/kg in dogs are shown in figure 14.

Pharmacokinetic profiles of GS-3 and its metabolites in plasma and PBMCs following intravenous administration of GS-3 at 1 mg/kg in dogs are shown in figure 15.

Pharmacokinetic profiles of GS-3 and its metabolites in plasma and PBMCs following oral administration of GS-3 at 4 mg/kg in dogs are shown in figure 16.

Example: Purification and Biochemical Characterization of GS-7340 Ester Hydrolase:

## Major Metabolites of GS-7340

**Metabolism of GS-7340:**

There is broad consensus that the bioactivation of nucleotide amide triesters follows a general scheme (Figure 1) (Valette, 1996; McGuigan, 1998a, 1998b; Saboulard, 1999; Siddiqui, 1999). Step A is the hydrolysis of the amino acid carboxylic ester. A nucleophilic attack by the carboxylic acid of the phosphorous (StepB) is believed to initiate the formation of the 5-membered cyclic intermediate which in turn is quickly hydrolyzed to the monoamidate diester (referred to as the amino acid nucleoside monophosphate, AAM, or metabolite X, Step C). This compound is considered an intracellular depot form of the antiviral nucleoside. Various enzymes as well as non-enzymatic catalysis have been implicated in Step D which is the hydrolysis of the amide bond resulting in the formation of the nucleotide. The nucleotide is activated by enzymatic phosphorylation to nucleotide di- and tri-phosphates.

In the case of GS-7340, the efficient conversion of this pro-drug to the amino acid nucleoside monophosphate (Metabolite X, Figure 2) is a necessary step for the observed accumulation of Metabolite X in peripheral blood mononuclear cells (PBMC). Purification of the Enzyme(s) responsible for the cleavage of GS-7340 amino acid carboxylic ester resulting in the formation of Metabolite X is the subject of this example.

#### **Ester Hydrolase Assay:**

The enzymatic production of metabolite X from GS-7340 was monitored using the following Ester Hydrolase assay: Varying amounts of peripheral blood mononuclear cell (PBMC) extracts, column fractions or pools were incubated with [ $^{14}$ C] GS-7340 at 37°C for 10 – 90 min. The production of [ $^{14}$ C] Metabolite X was monitored by measuring the amount of radioactivity retained on an anion exchange resin (DE-81). HPLC and mass spectrometry analysis of the reaction mixture and radioactivity retained on the filter confirmed that only [ $^{14}$ C]-Metabolite X bound the DE-81 filter. Under the assay conditions, the more hydrophobic [ $^{14}$ C] GS-7340 is not retained on the DE-81 membrane. The final reaction conditions were: 25 mM 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.5, 100mM NaCl, 1 mM DTT, 30  $\mu$ M [ $^{14}$ C] GS-7340, 0.1% NP40 and varying amounts of enzyme in a final volume of 60  $\mu$ l. The reaction mixture was incubated at 37°C and at 10, 30 and 90 minutes, 17 $\mu$ l of the reaction mixture was spotted onto a DE-81 filter. The filter was washed with 25mM Tris, pH 7.5 100mM NaCl, dried at room temperature, placed in vials containing 5ml of scintillation fluid. [ $^{14}$ C]-Metabolite X present on the filters was determined using a scintillation counter (LS 6500, Beckman, \_\_\_\_). Activity was expressed as pmoles Metabolite X produced / minute / volume enzyme sample. Ester Hydrolase Specific Activity was expressed as pmoles Metabolite X produced / minute /  $\mu$ g protein.

#### **Non-Specific Esterase Assay:**

Non-specific ester hydrolase activity was monitored by monitoring the enzymatic cleavage of alpha naphthyl acetate (ANA) (Mastropaolo, W and Yourno, J 1981). This substrate has been used for both the measurement esterase enzyme activity and *in situ* staining of esterases in tissue samples (Yourno, J and Mastropaolo, W. 1981; Yourno, J et Al 1981; Yourno, J et al 1986). The method described is a modification of the assay described by Mattes, P and Mattes, WB, 1992). Varying amounts of peripheral blood mononuclear cell (PBMC) extracts column fractions or pools were incubated with ANA at 37°C for 20 min. The final reaction

conditions were: 10 mM sodium phosphate, pH 6.5, 97  $\mu$ M ANA and varying amounts of enzyme in a final volume of 150  $\mu$ l. The reaction mixture was incubated at 37°C and at 20 minutes, and the reaction was stopped by the addition of 20  $\mu$ l of 10mM Blue salt RR in 10% sodium dodecyl sulfate (SDS). The alpha naphthyl-Blue salt RR product was detected by reading absorbance at 405nm. Activity was expressed as pmoles product produced /minute/ volume enzyme sample.

#### **Extraction of GS-7340 Ester Hydrolase from Human PBMCs:**

Fresh human PBMC were obtained from patients undergoing leukapheresis; cells were shipped in plasma and processed within 26 h of draw. PBMC cells were harvested by centrifugation at 1200 X g for 5 minutes and washed three times by re-suspension in RBC lysis buffer (155 mM  $\text{NH}_4\text{Cl}$ , 1 mM EDTA, 10mM  $\text{KHCO}_3$ ). Washed cells ( $29 \times 10^9$ ) were suspended in 150 ml of lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 20 mM  $\text{CaCl}_2$ , 1 mM DTT and 1% NP40) and incubated on ice for 20 minutes. The PBMC crude extract was centrifuged at 1000 X g for 30 min to remove unlysed cells and the supernatant at 100,000 X g for 1h. The 100,000 X g supernatant (PBMC Extract: P0) was harvested (165ml) and the pellets (1000 X g and 100,000 X g pellets) were resuspended in 10 mM Tris, pH 7.4, 150 mM NaCl, 20 mM  $\text{CaCl}_2$ , 1 mM DTT and assayed for GS-GS-7340 ester hydrolase activity. Assays showed that < 2% of the GS-GS-7340 Ester Hydrolase enzymatic activity was present in the pellets. The cell extract was snap frozen in liquid Nitrogen and stored at -70°C.

#### **Anion Exchange Chromatography:**

The PBMC Extract ( $15 \times 10^9$  cells, 75 – 85ml) was diluted 1:10, (vol: vol) with 25mM Tris, pH 7.5, 10% glycerol, 1mM DTT (Q15 Buffer A) and loaded onto an anion exchange column (2.5cm X 8.0 cm, Source Q15 (Amersham Biosciences)), previously equilibrated with Q15 Buffer A. Bound protein was eluted with a linear NaCl gradient (30 column volumes (CV)) to 0.5M NaCl. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (12.0 ml) were collected and assayed for both GS-7340 Ester Hydrolase and ANA Esterase activity. GS-7340 Ester Hydrolase activity eluted as a single major peak at 50 – 75 mM NaCl (Table 1). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 50 – 65% of total activity loaded. Significant ANA Esterase activity (30-40% of total activity loaded) was detected in the column FT; however, ~ 30% eluted in two peaks at 70 – 100 mM

NaCl (Table 1). Fractions containing GS-7340 Ester Hydrolase activity (Q15 pool) were pooled, snap frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

#### **Hydrophobic Interaction (HIC) Chromatography:**

The Q15 pool was defrosted and diluted 1:1, (vol: vol) with 25mM Tris, pH 8.0, 0.5 M  $(\text{NH}_4)_2\text{SO}_4$ , 1mM DTT, 10% glycerol BS-HIC Buffer A). 1M  $(\text{NH}_4)_2\text{SO}_4$  was added to yield a final concentration of 0.5M  $(\text{NH}_4)_2\text{SO}_4$  in the sample. The sample (300ml /  $10 \times 10^9$  cells) was loaded onto a Butyl Sepharose HIC column (5ml HiTrap, Amersham Biosciences) previously equilibrated with BS-HIC Buffer A. Bound protein was eluted with a linear gradient (15 CV) decreasing to with 25mM Tris, pH 8.0, 1mM DTT, 10% glycerol. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (4.0 ml) were collected and assayed for both GS-7340 Ester Hydrolase and ANA Esterase activity. GS-GS-7340 Ester Hydrolase activity eluted as a single major peak at 200 – 75 mM  $(\text{NH}_4)_2\text{SO}_4$  (Table 1). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 50 – 65% of total activity loaded (Table 1). Significant ANA Esterase activity (85% of total activity loaded) was detected in the column FT; however, ~ 10-15% eluted in a peak at 450 – 300 mM  $(\text{NH}_4)_2\text{SO}_4$ . Fractions containing GS-7340 Ester Hydrolase activity (BS-HIC pool) were pooled, snap frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

#### **Hydroxyapatite (HAP) Chromatography:**

The BS-HIC pool (40 ml /  $10 \times 10^9$  cells) was defrosted, concentrated to 2.0ml using a 10kDa molecular weight cutoff concentrator (20ml Vivaspin concentrator, Viva Science, Carlsbad, CA), and diluted to 20ml with 1mM sodium phosphate, pH 6.85, 10% glycerol, 1mM DTT (HAP Buffer A). The sample containing the GS-7340 Ester Hydrolase activity was loaded onto a HAP column (0.75 ml, 5mm X 20mm; ceramic hydroxyapatite, BioRad, Hercules, CA), previously equilibrated with HAP Buffer A. Bound protein was eluted with a 40 CV gradient to 500 mM sodium phosphate, pH 6.85, 10% glycerol, 1 mM DTT. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a single major peak at 70 -85 mM sodium phosphate (Table 1a). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 40 -45% of total activity loaded (Table 1a). Fractions containing GS-7340 Ester Hydrolase activity (HAP pool) were pooled, snap frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

**High Resolution Gel Filtration Chromatography:**

The BS-HIC pool (5ml /  $1.25 \times 10^9$  cells) was defrosted, concentrated to 0.05ml using a 5kDa molecular weight cutoff concentrator (20ml Vivaspin concentrator, Viva Science, Carlsbad, CA), and loaded onto a high resolution Gel Filtration column (8mm X 300mm, KW 802.5; Shodex, Thomas Instrument Co., Oceanside, CA), previously equilibrated with 25mM Tris, pH 7.5, 150mM NaCl, 10% glycerol, 20mM CaCl<sub>2</sub>, 1mM DTT (KW 802.5 column buffer). Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a single major peak at in fractions corresponding to an apparent molecular weight of 70 – 100 kDa (Table 1a). Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was >75% of total activity loaded (Table 1a). Fractions containing GS-7340 Ester Hydrolase activity (KW 802.5 pool) were pooled, snap frozen in liquid nitrogen and stored at -70°C.

**Summary of GS-7340 Ester Hydrolase Purification:**

The following table summarizes the purification of GS-7340 Ester Hydrolase achieved. Protein was measured by a Coomassie Blue stain colorimetric assay (Bradford Protein Assay, BioRad, Hercules, CA). The Specific Activity (pmoles Metabolite X produced / minute /  $\mu$ g protein) of the partially purified GS-7340 Ester Hydrolase varied from 666 to 1500. This represents a 222 – 750 fold purification from the PBMC extracts. Overall Recovery of GS-7340 Ester Hydrolase from PBMC extracts was approximately 10%.

**Table 1c: Purification Summary of GS-7340 Ester Hydrolase:**

| Sample name | PBMC        | Protein concentration (mg/ml) | Volume (ml) | Protein (mg) | Total Activity (pmol/min) | Specific Activity pmol/min/ $\mu$ g | % Recovery |
|-------------|-------------|-------------------------------|-------------|--------------|---------------------------|-------------------------------------|------------|
| P0 PBMC     | 30 X $10^9$ | 5.0                           | 200         | 1000         | 2.0 – 3.0 X $10^6$        | 2.0 – 3.0                           |            |
| Q15 Pool    |             | 0.116 – 0.167                 | 300         | 35 – 50      | 1.0 – 1.5 X $10^6$        | 20 – 42                             | ~50        |
| BS-HIC Pool |             | 0.02 – 0.035                  | 100         | 2.0 – 3.5    | 0.5 – 0.75 X $10^6$       | 142– 375                            | ~50        |
| HAP Pool    |             | 0.02 – 0.03                   | 10          | 0.2 – 0.3    | 0.2 – 0.3 x $10^6$        | 666 – 1500                          | ~40        |
|             |             |                               |             |              |                           | % Total Recovery                    | ~10        |

**Biochemical Characterization of GS-7340 Ester Hydrolase:**

Determination of the Isoelectric point (pI) of GS-7340 Ester Hydrolase:

The isoelectric point (pI) of a protein is defined as the pH at which the protein has no net ionic charge. Chromatofocusing is a chromatographic procedure in which a negatively charged protein is bound to a hydrophilic column with a net positive ionic charge. The protein is loaded at a pH 1 to 2 pH units higher than its estimated pI, and the bound protein is eluted by generating a decreasing pH gradient using a pH 3.0 to 4.0 buffer. The proteins will be eluted at a pH corresponding to pI.

An aliquot of the BS HIC pool (20 ml,  $5 \times 10^9$  cells) was concentrated to 4.0 ml and prepared for chromatofocusing chromatography by exchanging buffer using a desalting column. 1.0 ml aliquots of the concentrated BS HIC pool were loaded onto a 5.0 ml desalting column (5.0 ml HiTrap, Amersham Biosciences, Piscataway, NJ) previously equilibrated with 25mM ethanolamine, pH 7.8 (pH'd with iminodiacetic acid), 10% glycerol (Mono P Buffer A). The desalted GS-7340 Ester Hydrolase activity was loaded onto a chromatofocusing column (5mm X 5mm HR Mono P, Amersham Biosciences, Piscataway, NJ) previously equilibrated with Mono P Buffer A. Bound protein was eluted with a 20CV gradient to pH 3.6 with 10 ml / 100 ml Polybuffer 74 (Amersham Biosciences) pH'd to 4.0 with iminodiacetic acid. This

chromatofocusing protocol produces a linear pH gradient from pH 7.8 to pH 3.6. Eluting protein was detected by monitoring Absorbance at 280nm. Fractions (0.5 ml) were collected and assayed for GS-7340 Ester Hydrolase. GS-7340 Ester Hydrolase activity eluted as a single major peak at pH 5.5 to 4.5. Recovery of Total GS-7340 Ester Hydrolase activity in the eluted fractions was 65 -70% of total activity loaded. Fractions containing GS-7340 Ester Hydrolase activity (KW 802.5 pool) were pooled, snap frozen in liquid nitrogen and stored at  $-70^{\circ}\text{C}$ .

#### **Inhibition of GS-7340 Ester Hydrolases by Serine Hydrolase Inhibitors:**

Fluorophosphonate / fluorophosphate (Diisopropylfluorophosphate (DFP)) derivatives, isocoumarins such as 3,4 dichloroisocoumarin (3,4-DCI) and peptide carboxyl esters of chloro- and fluoro-methyl ketones (AlaAlaProAla-CMK, AlaAlaProVal-CMK, PheAla-FMK) are known effective inhibitors of serine hydrolases (Powers and Harper 1986; Delbaere and Brayer, 1985; Bullock et al 1996; Yongsheng et al 1999; Kam et al 1993). Inhibition of the enzymatic production of metabolite X from GS-7340 was monitored using the following Ester Hydrolase Inhibition assay: Varying amounts of partially purified GS-7340 Ester Hydrolase and control enzymes (human leukocyte elastase (huLE), porcine liver carboxylesterase (PLCE)) were incubated with [ $^{14}\text{C}$ ] GS-7340 in the presence and absence of varying amounts of known serine hydrolase inhibitors at  $37^{\circ}\text{C}$  for 10 – 90 min. The production of [ $^{14}\text{C}$ ] Metabolite X was monitored by measuring the amount of radioactivity retained on an anion exchange resin (DE-81). The final reaction conditions were: 25 mM 2-[N-morpholino]ethanesulfonic acid (MES), pH 6.5, 100mM NaCl, 1 mM DTT, 30  $\mu\text{M}$  [ $^{14}\text{C}$ ] GS-7340, 0.1% NP40 varying amounts of enzyme and inhibitors (1.0 $\mu\text{M}$  – 1mM) in a final volume of 60  $\mu\text{l}$ . The reaction mixture was incubated at  $37^{\circ}\text{C}$  and at 10, 30 and 90 minutes, 17 $\mu\text{l}$  of the reaction mixture was spotted onto a DE-81 filter. The filter was processed and the amount of [ $^{14}\text{C}$ ] Metabolite X present was determined as described above. Activity was expressed as pmoles Metabolite X produced / minute / volume enzyme sample. Inhibition of Ester Hydrolase and control hydrolases was expressed as percent activity present at a given concentration of inhibitor compared to hydrolase activity in the absence of the inhibitor. The results of the inhibition experiments are shown in Table 2A/B. The serine hydrolase inhibitors, 3,4-DCI and DFP inhibit GS-7340 Ester Hydrolase with estimated IC<sub>50</sub>'s of 4.0 and 30  $\mu\text{M}$ , respectively. The peptide chloro- and fluoro-methyl ketones are less effective inhibitors with estimated IC<sub>50</sub>'s of 100 –400  $\mu\text{M}$  (Table 2 A / B).



**Table 2A: Inhibition of GS-7340 Ester Hydrolase and Control Enzymes by Serine Hydrolase Inhibitors**

| Inhibitor                  | IC50 ( $\mu$ M)               |       |      |
|----------------------------|-------------------------------|-------|------|
|                            | GS-7340<br>Ester<br>Hydrolase | PLCE  | huLE |
| 3,4-dichloroisocoumarin    | 4.0                           | 250   | 3.0  |
| MeOSuC-Ala-Ala-Pro-Ala-CMK | 200-400                       | >1000 | 60   |
| MeOSuc-Ala-Ala-Pro-Val-CMK | 100                           | >1000 | 4.0  |
| Biotin-Phe-Ala-FMK         | 100                           | >1000 | 100  |
| DFP                        | 30                            | 0.05  | -    |

**Table 2B: Inhibition of GS-7340 Ester Hydrolase and Control Enzymes by Serine Hydrolase Inhibitors**

|                                | <b>Inhibitor<br/>(<math>\mu</math>M)</b> | <b>Relative Activity<br/>(%)</b> | <b>IC50<br/>(<math>\mu</math>M)</b> |
|--------------------------------|------------------------------------------|----------------------------------|-------------------------------------|
| <b>GS-7340 Ester Hydrolase</b> |                                          |                                  |                                     |
| <b>3,4-dichloroisocoumarin</b> | 1.0                                      | 100                              | 4.0                                 |
|                                | 10                                       | 25                               |                                     |
|                                | 100                                      | 5                                |                                     |
|                                | 1000                                     | <2                               |                                     |
| <b>DFP</b>                     | 1.0                                      | 100                              | 30-40                               |
|                                | 10                                       | 90                               |                                     |
|                                | 100                                      | 35                               |                                     |
|                                | 1000                                     | <2                               |                                     |
| <b>Biotin-Phe-Ala-FMK</b>      | 1.0                                      | 100                              | 100                                 |
|                                | 10                                       | 95                               |                                     |
|                                | 100                                      | 50                               |                                     |
|                                | 1000                                     | <2                               |                                     |
| <b>PLCE</b>                    |                                          |                                  |                                     |
| <b>3,4-dichloroisocoumarin</b> | 1.0                                      | 100                              | 250                                 |
|                                | 10                                       | 100                              |                                     |
|                                | 100                                      | 90                               |                                     |
|                                | 1000                                     | 20                               |                                     |
| <b>DFP</b>                     | 0.001                                    | 100                              | 0.05                                |
|                                | 0.01                                     | 90                               |                                     |
|                                | 0.1                                      | 20                               |                                     |
|                                | 1.0                                      | <2                               |                                     |
| <b>Biotin-Phe-Ala-FMK</b>      | 1.0                                      | 100                              | >1000                               |
|                                | 10                                       | 100                              |                                     |
|                                | 100                                      | 100                              |                                     |
|                                | 1000                                     | 80                               |                                     |
| <b>huLE</b>                    |                                          |                                  |                                     |
| <b>3,4-dichloroisocoumarin</b> | 1.0                                      | 100                              | 4.0                                 |
|                                | 10                                       | 25                               |                                     |
|                                | 100                                      | 5                                |                                     |
|                                | 1000                                     | <2                               |                                     |
| <b>Biotin-Phe-Ala-FMK</b>      | 1.0                                      | 100                              | 100                                 |
|                                | 10                                       | 93                               |                                     |
|                                | 100                                      | 48                               |                                     |
|                                | 1000                                     | <2                               |                                     |

**Summary of Biochemical Characterization of GS-7340 Ester Hydrolase:**

Summarizing, GS-7340 Ester Hydrolase is a novel enzyme characterized by being capable of being recovered from human PBMCs by a process comprising

- (a) lysing human PBMCs;
- (b) extracting the lysed cells with detergent;
- (c) separating the solids from supernatant and recovering the supernatant;
- (d) contacting the supernatant with an anion exchange medium;
- (e) eluting the Hydrolase from the anion exchange medium;
- (f) contacting the eluate with a hydrophobic chromatographic medium; and
- (g) eluting the Hydrolase from the hydrophobic chromatographic medium.

GS-7340 Ester Hydrolase is useful in screening candidate compounds to assess the likelihood that they can be processed to form depot metabolites in lymphoid tissue. The candidates are assayed in the same fashion as described herein for GS-7340, taking into account differences in the nature of the suspected substrate as will be apparent to the ordinary artisan.

GS-7340 Ester Hydrolase optionally is labelled with a detectable group such as a radiolabel or covalently bound to an insoluble matrix such as Sepharose using techniques heretofore employed for other enzymes having similar properties, as will be apparent to the ordinary artisan.

GS-7340 Ester Hydrolase has the following properties:

- 1) GS-7340 Ester Hydrolase can be partially purified from fresh PBMC Extracts: SA = 666 -1500 pmoles MetX/min/ug protein.
- 2) GS-7340 Ester Hydrolase can be separated from non-specific Esterases capable of cleaving alpha-naphthyl acetate (ANA), a non-specific substrate shown to be cleaved by many carboxylesterases and hydrolases.
- 3) Multiple GS-7340 Ester Hydrolase activity peaks are not eluted from columns during purification.

- 4) The MW of GS-7340 Ester Hydrolase on Gel Filtration is ~ 70 - 100kDa
- 5) The pI of GS-7340 Ester Hydrolase is pH 4.5 -5.5
- 6) Evidence to date suggests that the SA of isolated GS-7340 Ester Hydrolase is likely to be > 10,000.
- 7) The serine hydrolase inhibitors, 3,4-DCI and DFP inhibit GS-7340 Ester Hydrolase with estimated IC<sub>50</sub>'s of 4.0 and 30  $\mu$ M, respectively. The peptide chloro- and fluoro-methyl ketones are less effective inhibitors with estimated IC<sub>50</sub>'s of 100 –400  $\mu$ M (Table 2 A / B).

**References:**

- Bullock, TL et al 1996 J Mol Biol 255: 714-725.
- Delbaere, LT and Brayer, GD 1985 J Mol Biol 183:89-103
- Kam C et al 1993 Bioconjugate Chem 4: 560-567
- Mastropaolo, W and Yourno, J 1981 Analytical Chemistry 115: 188-193
- Mattes, PM, and Mattes, WB, 1992. Toxicol. Appl. Pharmacol. 114:71-76
- McGuigan, CPW et al 1998a Antiviral Chem and Chemotherapy 9: 109-115
- McGuigan, CPW et al 1998b Antiviral Chem and Chemotherapy 9: 473-479
- Powers, JC and Harper, JW 1986 Inhibitors of serine proteinases. In Proteinase Inhibitors (AJ Barrett and G Salvesen, Eds.) Elsevier, Amsterdam, New York, Oxford, pp55-152)
- Saboulard, DL et al 1999 Molec Pharmacol 56:693-704
- Siddiqui, AQC and McGuigan, CPW 1999 J Med Chem 42:4122-4128
- Valette, GA et al 1996 J Med Chem 39:1981-1990
- Yongsheng, the linker et al 1999 Proc Natl Acad Sci 96:14694-14699
- Yourno, J and Mastropaolo, W. 1981 Blood, 58:939-945
- Yourno, J et al 1981. Blood, 60: 24-29
- Yourno, J et al 1986 J Histochem and Cytochem 34:727-33)

Example: Candidate Compounds

A large number of examples describing the preparation of candidate compounds active against HIV protease, HIV integrase and HIV polymerase (non-nucleotide reverse transcriptase inhibitors, or NNRTIs) are found in copending applications and are set forth below. These compounds are examples of candidate compounds that are typical of those which are suitable for use in the method and libraries of this invention.

Incorporation by Reference

All publications and patent applications cited herein are incorporated by reference to the same extent as if the full text of each individual publication or patent application was contained herein. The incorporated text will be apparent from context if not specifically set forth. Incorporated by reference are (a) US patent applications 60/373,533 and 60/375,665

hereof based on such applications and (b) U.S. patent application 60/375,622 (attorney docket 260.P) and the section 111(a) application filed of even date hereof based on such application. Further, the content of PCT/US 03/12901 and PCT/US 03/12926 and in particular all embodiments thereof relating to the claims herein are incorporated by reference in their entirety.

## WE CLAIM:

1. A method comprising
  - (a) identifying a non-nucleotide prototype compound;
  - (b) substituting the prototype compound with a phosphonate-containing group to produce a candidate compound; and
  - (a) determining the anti-HIV activity of the candidate compound.
2. A method comprising
  - (a) selecting a non-nucleotide candidate compound containing at least one esterified carboxyl or esterified phosphonate-containing group; and
  - (b) determining the intracellular persistence of the candidate compound or a esterolytic metabolite of the esterified carboxyl or phosphonate-containing group thereof.
3. The method of claim 1 wherein the tissue selectivity of the candidate compound and/or at least one of its intracellular depot metabolites is determined.
4. The method of claim 1 wherein the intracellular residence time of said candidate compound and/or at least one of its intracellular depot metabolites is determined.
5. The method of claim 2 comprising additionally determining the activity of at least one of said metabolites against HIV protease.
6. The method of claim 2 wherein the metabolite is a carboxylic acid.
7. The method of claims 1 or 2 comprising determining the ability of the candidate to inhibit HIV.
8. The method of claim 1 wherein the prototype is already known to have therapeutic activity against HIV.

9. The method of claim 2 comprising selecting and determining the intracellular persistence of a plurality of candidate compounds.
10. The method of claims 1 or 2 wherein compounds which are not candidate compounds are tested in parallel together with at least one candidate compound.
11. The method of claim 2 comprising determining cleavage of one or more candidates by GS-7340 Ester Hydrolase.
12. The method of claims 1 or 2 wherein the candidate is an amino acid phosphonoamidate in which a carboxyl of the amino acid is esterified.
13. The method of claim 1 wherein the prototype compound is known to inhibit HIV protease, HIV integrase or HIV reverse transcriptase.
14. The method of claim 1 wherein the prototype compound is not known to be an analogue of a naturally occurring phosphate-containing enzyme substrate.
15. The method of claim 1 wherein the prototype compound is not a nucleoside.
16. The method of claim 1 wherein the prototype compound does not contain a nucleoside base.
17. The method of claim 1 wherein an intracellular depot metabolite is tested.
18. The method of claim 1 also comprising determining the resistance of HIV to the candidate compound and/or its intracellular depot metabolite.
19. The method of claim 1 comprising determining the tissue selectivity and/or intracellular residence time for a first candidate compound and/or its intracellular depot metabolite, preparing or selecting additional analogues of said first candidate compound, and determining the therapeutic activity of said additional analogues without determining tissue selectivity and/or intracellular residence time of said analogues.
20. The method of claim 1 comprising determining the safety and/or anti-HIV therapeutic activity of the candidate compound in *in vitro* cell culture, in enzyme assay, in animals or in humans.



21. The method of claim 1 wherein the prototype compound is a pharmaceutical product licensed by the US Food and Drug Administration.
22. The method of claim 1 wherein the prototype compound is one which is disclosed to have anti-HIV activity in a patent or published patent application on or before the filing date of this application.
23. The method of claim 1 comprising determining susceptibility to hydrolysis of the carboxyl or phosphonate esters by GS-7340 Ester Hydrolase, said Hydrolase characterized by being capable of being recovered from human PBMCs by a process comprising
- (b) lysing human PBMCs;
  - (c) extracting the lysed cells with detergent;
  - (d) separating the solids from supernatant and recovering the supernatant;
  - (e) contacting the supernatant with an anion exchange medium;
  - (f) eluting the Hydrolase from the anion exchange medium;
  - (g) contacting the eluate with a hydrophobic chromatographic medium; and
  - (h) eluting the Hydrolase from the hydrophobic chromatographic medium.
24. The method of claim 23 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa, has a pI of about 4.5-5.5 by chromatofocusing, is inhibited by 3,4 dichloroisocoumarin, binds to Butyl Sepharose HIC, binds to anion exchange medium Q15, and is capable of being recovered from human PBMCs.
25. The method of claim 2 wherein the intracellular residence time is determined as the half-life of at least one intracellular depot metabolite within a lymphoid tissue.
26. The method of claim 25 wherein the lymphoid tissue is PBMCs, helper cells, killer cells or lymph nodes.
27. The method of claim 1 wherein determining anti-HIV activity is by *in vitro* assay.
28. The method of claim 27 wherein the assay is conducted in an animal model or clinical trials.

29. The method of claims 1 or 2 comprising the additional steps of identifying a clinical trial compound from the final step, entering into clinical trials with said clinical trial compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.

30. The method of claim 29 wherein the clinical trial compound is not identical to the candidate compound

31. The method of claim 2 wherein intracellular persistence was determined by clinical studies comprising determination of the amount and timing of dosing of the candidate compound.

32. The method of claim 2 wherein the metabolite is intracellularly sequestered in PBMCs.

33. The method of claim 2 wherein greater than one metabolite is tested to determine intracellular residence time.

34. The method of claim 2 wherein the intracellular persistence is determined in PBMCs.

35. The method of claim 2 wherein the metabolite comprises the phosphonate group of Metabolite X.

36. The method of claim 2 wherein the metabolite comprises an unesterified carboxyl group.

37. The method of claim 2 wherein the intracellular depot metabolite comprises the group -P(O)(OH)-.

38. A library of candidate non-nucleotide anti-HIV compounds comprising a plurality of candidate compounds suspected to have anti HIV activity which contain esterified carboxyl or esterified phosphonate groups.

39. A library of candidate anti-HIV compounds which does not consist solely of nucleotides and which comprises a plurality of candidate compounds suspected to have anti-HIV activity which contain esterified carboxyl or esterified phosphonate groups.

40. The library of claims 38 or 39 comprising at least about 10 candidate compounds.
41. The library of claims 38 or 39 wherein the candidate compounds comprise (a) a phosphonate substituted with an amino acid or an organic acid, or (b) an amino acid, at least one of the carboxyl groups of the amino acid or organic acid being esterified.
42. The library of claims 38 or 39 wherein the compounds in the library are stored in discrete containers.
43. A method comprising testing the library of claims 39, 40, 41, or 42 to determine the anti-HIV activity of at least one candidate compound in the library.
44. The method of claim 43 comprising determining for tissue selectivity and/or the intracellular persistence of at least one of said candidate compounds and/or at least one of their intracellular metabolites.
45. The method of claim 43 comprising the additional steps of identifying a clinical trial compound from said library, entering into clinical trials with said clinical trial compound, obtaining regulatory approval to market said clinical trial compound for the treatment of HIV, and selling said clinical trial compound after said regulatory approval.
46. Isolated GS-7340 Ester Hydrolase.
47. The Hydrolase of claim 46 which is purified to a single major band on gel filtration chromatography.
48. The Hydrolase of claim 46 which is capable of being recovered from human PBMC cells.
49. The Hydrolase of claim 48 wherein the Hydrolase has a MW on gel filtration chromatography of about 70-100 kDa.
50. The Hydrolase of claim 50 which has a pI of about 4.5-5.5 by chromatofocusing
51. The Hydrolase of claim 50 which is inhibited by 3,4 dichloroisocoumarin,
52. The Hydrolase of claim 51 which binds to Butyl Sepharose HIC.
53. The Hydrolase of claim 52 which binds to anion exchange medium Q15.

54. The Hydrolase of claim 53 which binds to hydroxyapatite.
55. The Hydrolase of claim 46 which is cross-linked to an insoluble medium.
56. A method comprising obtaining a substantially pure organic molecule, optionally contacting the organic molecule with another molecule to produce a composition, and contacting the Hydrolase of claim 46 with said organic molecule or composition.
57. The method of claim 56 wherein the organic molecule is an anti-HIV compound.
58. A method comprising contacting GS-7340 Ester Hydrolase with an organic compound in an *in vitro* or cell culture environment.
59. The method of claim 58 wherein the environment is cell free.
60. A composition comprising a substantially pure organic compound and isolated GS-7340 Ester Hydrolase.
61. A composition comprising an organic compound and GS-7340 Ester Hydrolase in an *in vitro* or cell culture environment.
62. In a method for identifying an anti-HIV therapeutic compound, the improvement comprising substituting a prototype compound with an esterified phosphonate or esterified carboxyl group to produce a candidate compound and assaying the resulting candidate compound for its anti-HIV activity.
63. The method of claim 61 wherein the candidate is assayed for its intracellular persistence.
64. The method of claim 63 wherein the candidate is assayed for its extracellular stability against hydrolysis of the carboxyl or phosphonate ester.
65. The method of claim 64 comprising selecting from a plurality of candidates a candidate which is esterolytically cleaved intracellularly to yield an intracellular persistent metabolite having anti-HIV activity and which candidate is substantially esterolytically stable against extracellular hydrolysis of the carboxyl or phosphonate ester.
66. The method of claim 65 wherein the candidate is substantially stable against hydrolysis of the carboxyl or phosphonate ester outside of the cell.

67. The method of claim 62 wherein the candidate is substituted with a phosphonate group comprising monosubstitution with (a) an amino acid linked through an amino group to the phosphorus atom or (b) an organic acid, and wherein a carboxylic acid of the amino acid or organic acid is esterified.
68. The method of claim 62 wherein the candidate is substituted with a group comprising an amino acid, wherein a carboxylic acid of the amino acid is esterified.
69. The method of claim 68 wherein the carboxylic acid is the residue of a hydroxyorganic acid linked to the phosphorus atom through an oxygen atom.
70. The method of claims 68 or 69 wherein the hydroxy group of the hydroxyorganic acid or the amino group of the amino acid are in the alpha position.
71. A method for identifying a candidate compound as a suitable pro-drug, comprising:
- (a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;
  - (b) contacting the candidate compound with an extract capable of catalyzing the hydrolysis of a carboxylic ester; and
  - (c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.
72. The method of claim 71, wherein said extract is obtained from peripheral blood mononuclear cells.
73. A method for identifying a candidate compound as a suitable pro-drug, comprising:
- (a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;

(b) contacting the candidate compound with an extract of peripheral blood mononuclear cells having carboxylic ester hydrolase activity to produce a metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

74. The method of claim 73, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

75. The method of claim 74, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

76. The method of claim 73, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

77. The method of claim 73, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

78. The method of claim 73, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound.

79. The method of claim 73, further comprising (d) determining the intracellular persistence of the candidate compound.

80. The method of claim 73, further comprising (d) determining the intracellular persistence of the metabolite compound.

81. The method of claim 73, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

82. The method of claim 73, further comprising (d) determining the tissue selectivity of the candidate compound.

83. The method of claim 73, further comprising (d) determining the tissue selectivity of the metabolite compound.
84. The method of claim 73, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.
85. The method of claim 73, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.
86. The method of claim 73, further comprising (d) determining the HIV-inhibition ability of the candidate compound.
87. The method of claim 73, further comprising (d) determining the resistance of HIV to the candidate compound.
88. The method of claim 73, further comprising (d) determining the resistance of HIV to the metabolite compound.
89. The method of claim 73, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.
90. The method of claim 73, further comprising (d) determining the intracellular residence time of the candidate compound.
91. The method of claim 73, further comprising (d) determining the intracellular residence time of the metabolite compound.
92. The method of claim 73, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.
93. The method of claim 90, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.
94. The method of claim 91, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

95. The method of claim 92, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

96. The method of claim 93, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

97. The method of claim 94, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

98. The method of claim 95, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

99. The method of claim 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

100. The method of claim 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

101. The method of claim 73, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

102. The method of claim 101, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

103. A method for identifying a candidate compound as a suitable pro-drug, comprising:

- (a) providing the candidate compound having an esterified phosphonate group;
- (b) contacting the candidate compound with GS-7340 Ester Hydrolase to produce a metabolite compound; and



(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound.

104. The method of claim 103, wherein said providing step further comprises monosubstitution of the esterified phosphonate group with an organic acid having an esterified carboxyl group.

105. The method of claim 103, wherein said providing step further comprises monosubstitution of the esterified phosphonate group with an amino acid linked through an amino group to the phosphorus atom, wherein the amino acid has an esterified carboxyl group.

106. The method of claim 103, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

107. The method of claim 106, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

108. The method of claim 103, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

109. The method of claim 103, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

110. The method of claim 103, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound

111. The method of claim 103, further comprising (d) determining the intracellular persistence of the candidate compound.

112. The method of claim 103, further comprising (d) determining the intracellular persistence of the metabolite compound.

113. The method of claim 103, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.
114. The method of claim 103, further comprising (d) determining the tissue selectivity of the candidate compound.
115. The method of claim 103, further comprising (d) determining the tissue selectivity of the metabolite compound.
116. The method of claim 103, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.
117. The method of claim 103, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.
118. The method of claim 103, further comprising (d) determining the HIV-inhibition ability of the candidate compound.
119. The method of claim 103, further comprising (d) determining the resistance of HIV to the candidate compound.
120. The method of claim 103, further comprising (d) determining the resistance of HIV to the metabolite compound.
121. The method of claim 103, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.
122. The method of claim 103, further comprising (d) determining the intracellular residence time of the candidate compound.
123. The method of claim 103, further comprising (d) determining the intracellular residence time of the metabolite compound.
124. The method of claim 103, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.
125. The method of claim 122, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

126. The method of claim 123, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.
127. The method of claim 124, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.
128. The method of claim 125, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.
129. The method of claim 126, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.
130. The method of claim 127, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.
131. The method of claim 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.
132. The method of claim 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.
133. The method of claim 103, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.
134. The method of claim 133, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.
135. A method for identifying a candidate compound as a suitable pro-drug, comprising:
- (a) providing the candidate compound having an esterified carboxyl group;

(b) contacting the candidate compound with GS-7340 Ester Hydrolase to produce an metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

136. The method of claim 135, wherein said providing step comprises providing a candidate compound substituted with an amino acid group, wherein the amino acid has an esterified carboxyl group.

137. The method of claim 135, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

138. The method of claim 137, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

139. The method of claim 135, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

140. The method of claim 135, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

141. The method of claim 135, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound

142. The method of claim 135, further comprising (d) determining the intracellular persistence of the candidate compound.

143. The method of claim 135, further comprising (d) determining the intracellular persistence of the metabolite compound.

144. The method of claim 135, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

145. The method of claim 135, further comprising (d) determining the tissue selectivity of the candidate compound.
146. The method of claim 135, further comprising (d) determining the tissue selectivity of the metabolite compound.
147. The method of claim 135, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.
148. The method of claim 135, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.
149. The method of claim 135, further comprising (d) determining the HIV-inhibition ability of the candidate compound.
150. The method of claim 135, further comprising (d) determining the resistance of HIV to the candidate compound.
151. The method of claim 135, further comprising (d) determining the resistance of HIV to the metabolite compound.
152. The method of claim 135, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.
153. The method of claim 135, further comprising (d) determining the intracellular residence time of the candidate compound.
154. The method of claim 135, further comprising (d) determining the intracellular residence time of the metabolite compound.
155. The method of claim 135, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.
156. The method of claim 153, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

157. The method of claim 154, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

158. The method of claim 155, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

159. The method of claim 156, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

160. The method of claim 157, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

161. The method of claim 158, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

162. The method of claim 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

163. The method of claim 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

164. The method of claim 135, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

165. The method of claim 164, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

166. A method for identifying a candidate compound as a suitable pro-drug, comprising:

(a) providing the candidate compound having an esterified phosphonate group or an esterified carboxyl group;

(b) contacting the candidate compound with an extract of peripheral blood mononuclear cells which has carboxylic ester hydrolase activity but does not cleave alpha-naphthyl acetate, to produce a metabolite compound; and

(c) identifying the candidate compound as a suitable pro-drug if the metabolite compound has a phosphonic acid group instead of the esterified phosphonate group of the candidate compound, or a carboxylic acid group instead of the esterified carboxyl group of the candidate compound.

167. The method of claim 166, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound known to have anti-HIV therapeutic activity with an esterified phosphonate or carboxyl group.

168. The method of claim 167, wherein said prototype compound is not a nucleoside, and does not contain a nucleoside base.

169. The method of claim 166, wherein said providing step comprises providing a candidate compound that is an amino acid phosphonoamidate, wherein a carboxyl group of the amino acid is esterified.

170. The method of claim 166, wherein said providing step comprises providing a candidate compound that is substantially stable against extracellular hydrolysis of the esterified group.

171. The method of claim 166, wherein said providing step comprises providing a candidate compound formed by substituting a prototype compound

172. The method of claim 166, further comprising (d) determining the intracellular persistence of the candidate compound.

173. The method of claim 166, further comprising (d) determining the intracellular persistence of the metabolite compound.

174. The method of claim 166, further comprising (d) determining the intracellular persistence of the candidate compound and the metabolite compound.

175. The method of claim 166, further comprising (d) determining the tissue selectivity of the candidate compound.

176. The method of claim 166, further comprising (d) determining the tissue selectivity of the metabolite compound.

177. The method of claim 166, further comprising (d) determining the tissue selectivity of the candidate compound and the metabolite compound.

178. The method of claim 166, further comprising (d) determining the anti-HIV protease activity of the metabolite compound.

179. The method of claim 166, further comprising (d) determining the HIV-inhibition ability of the candidate compound.

180. The method of claim 166, further comprising (d) determining the resistance of HIV to the candidate compound.

181. The method of claim 166, further comprising (d) determining the resistance of HIV to the metabolite compound.

182. The method of claim 166, further comprising (d) determining the resistance of HIV to the candidate compound and the metabolite compound.

183. The method of claim 166, further comprising (d) determining the intracellular residence time of the candidate compound.

184. The method of claim 166, further comprising (d) determining the intracellular residence time of the metabolite compound.

185. The method of claim 166, further comprising (d) determining the intracellular residence time of the candidate compound and the metabolite compound.

186. The method of claim 183, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

187. The method of claim 184, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.



188. The method of claim 185, wherein said step of determining the intracellular residence time of the metabolite compound comprises determining the half-life of the metabolite compound within lymphoid tissue.

189. The method of claim 186, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

190. The method of claim 187, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

191. The method of claim 188, wherein said step of determining the half-life of the metabolite compound further comprises determining the half-life of the metabolite compound within helper cells, killer cells, lymph nodes, or peripheral blood mononuclear cells.

192. The method of claim 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a cell-free environment.

193. The method of claim 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in vitro.

194. The method of claim 166, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in cell culture.

195. The method of claim 194, wherein said contacting step comprises contacting the candidate compound with GS-7340 Ester Hydrolase in a culture of peripheral blood mononuclear cells.

196. A candidate compound identified by the method of claim 71, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

197. A candidate compound identified by the method of claim 103, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

198. A candidate compound identified by the method of claim 134, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

199. A candidate compound identified by the method of claim 164, wherein the candidate compound is an amino acid phosphonoamidate in which a carboxyl group of the amino acid is esterified.

200. A candidate compound identified by the method of claim 71, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

201. A candidate compound identified by the method of claim 103, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

202. A candidate compound identified by the method of claim 134, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

203. A candidate compound identified by the method of claim 164, wherein the candidate compound is substituted with an amino acid group in which a carboxyl group of the amino acid is esterified.

204. The candidate compound of claim 200, wherein the amino group of the amino acid is in the alpha position.

205. The candidate compound of claim 201, wherein the amino group of the amino acid is in the alpha position.

206. The candidate compound of claim 202, wherein the amino group of the amino acid is in the alpha position.

207. The candidate compound of claim 203, wherein the amino group of the amino acid is in the alpha position.

208. A candidate compound identified by the method of claim 71, wherein the esterified phosphonate group is monosubstituted with a hydroxyorganic acid linked to the phosphorus atom through an oxygen atom.

209. The candidate compound of claim 133, wherein the hydroxy group of the hydroxyorganic acid is in the alpha position.

210. A candidate compound identified by the method of claim 71, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

211. A candidate compound identified by the method of claim 103, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

212. A candidate compound identified by the method of claim 134, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

213. A candidate compound identified by the method of claim 164, wherein the candidate compound is substantially stable against extracellular hydrolysis of the esterified group.

214. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of claim 71;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

215. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of claim 103;
- (b) determining the anti-HIV activity of the candidate compound; and

(c) determining the intracellular persistence of the candidate compound.

216. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of claim 134;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

217. A method of screening candidate compounds for suitability as anti-HIV therapeutic agents, comprising:

- (a) providing a candidate compound identified by the method of claim 164;
- (b) determining the anti-HIV activity of the candidate compound; and
- (c) determining the intracellular persistence of the candidate compound.

218. The method of claim 214, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

219. The method of claim 215, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

220. The method of claim 216, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

221. The method of claim 217, wherein said step (b) comprises determining the activity of the candidate compound against HIV protease.

222. The method of claim 214, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

223. The method of claim 215, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

224. The method of claim 216, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

225. The method of claim 217, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV.

226. The method of claim 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

227. The method of claim 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

228. The method of claim 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

229. The method of claim 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV protease.

230. The method of claim 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

231. The method of claim 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

232. The method of claim 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

233. The method of claim 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV integrase.

234. The method of claim 222, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

235. The method of claim 223, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

236. The method of claim 224, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

237. The method of claim 225, wherein said step (b) comprises determining the ability of the candidate compound to inhibit HIV reverse transcriptase.

238. The method of claim 214, wherein said step (b) further comprises determining the resistance of HIV to the candidate compound.

239. The method of claim 214, wherein said step (b) is performed by *in vitro* assay.

240. The method of claim 214, wherein said step (b) further comprises determining the anti-HIV activity of an acid metabolite of the candidate compound.

241. The method of claim 240, wherein said acid metabolite is a carboxylic acid compound formed by esterolytic hydrolysis of the candidate compound.

242. The method of claim 240, wherein said acid metabolite is a phosphonic acid compound formed by esterolytic hydrolysis of the candidate compound.

243. The method of claim 214, wherein said step (c) comprises determining the intracellular residence time of the candidate compound.

244. The method of claim 214, wherein said step (c) further comprises determining the intracellular residence time of an acid metabolite of the candidate compound.

245. The method of claim 244, wherein said acid metabolite is a carboxylic acid compound formed by esterolytic hydrolysis of the candidate compound.

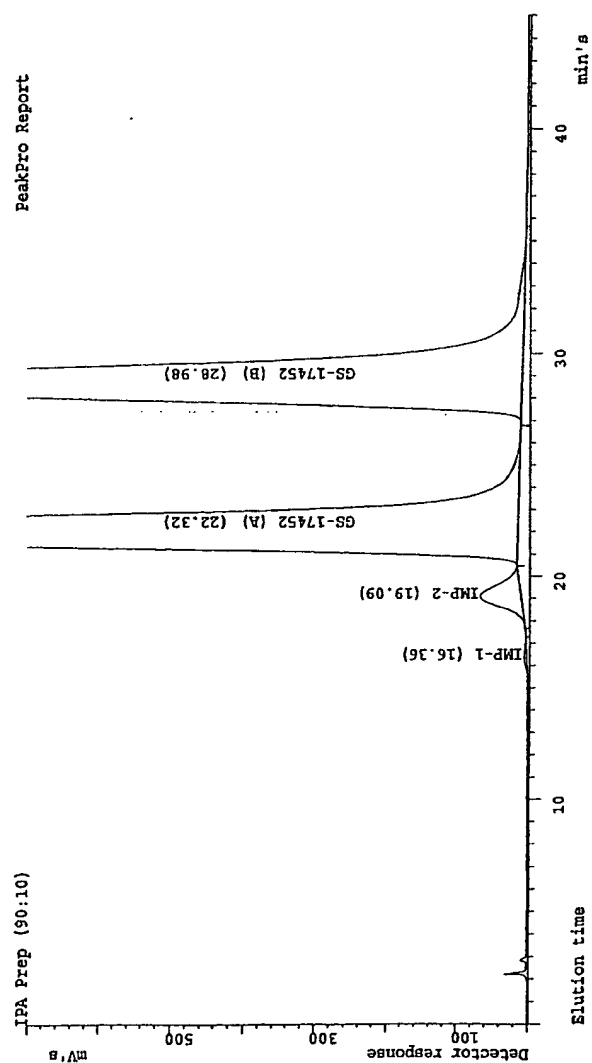
246. The method of claim 244, wherein said acid metabolite is a phosphonic acid compound formed by esterolytic hydrolysis of the candidate compound.

247. The method of claim 244, wherein said step (c) further comprises determining the half-life of the metabolite compound within lymphoid tissue.

248. The method of claim 247, wherein in said step of determining the half-life of the metabolite compound within lymphoid tissue, the lymphoid tissue is selected from the group consisting of helper cells, killer cells, lymph nodes, and peripheral blood mononuclear cells.

249. The method of claim 214, further comprising (d) determining the tissue selectivity of the candidate compound.

250. The method of claim 249, wherein said step (d) further comprises determining the tissue selectivity of an acid metabolite of the candidate compound.

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Fig. 1

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Fig. 2

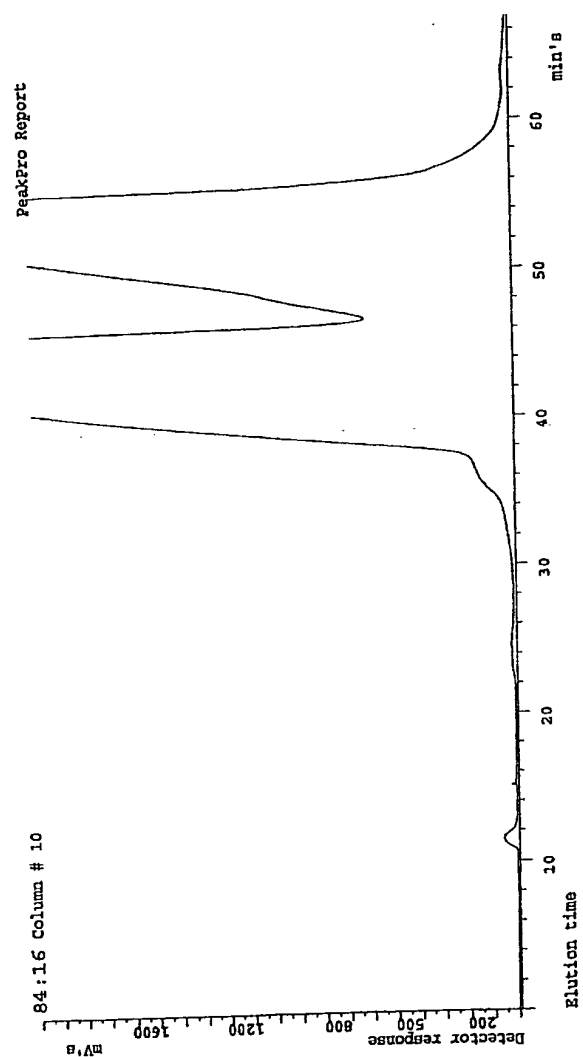




Fig. 3

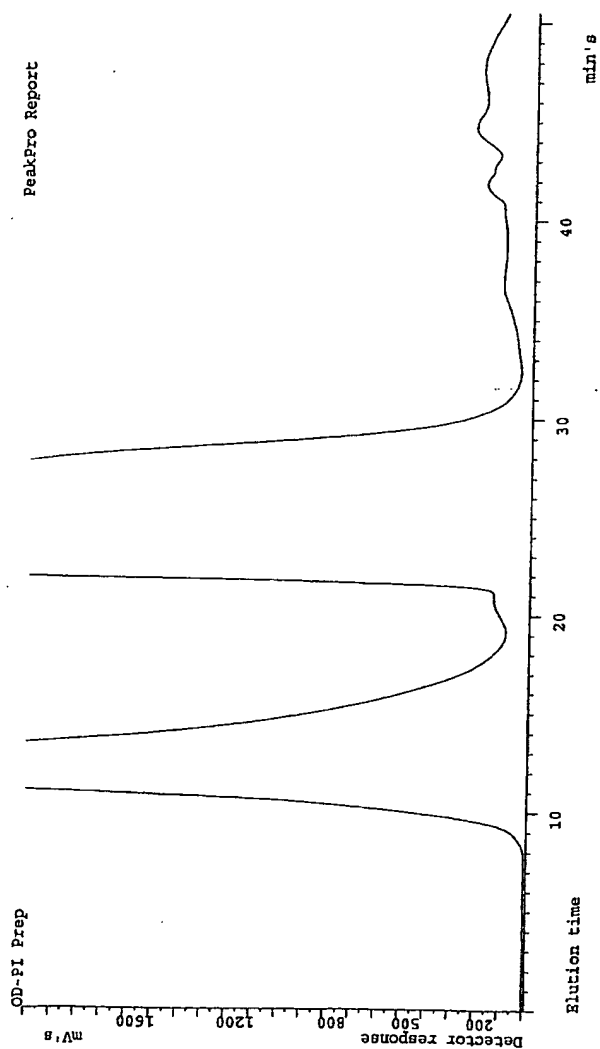


Fig. 4

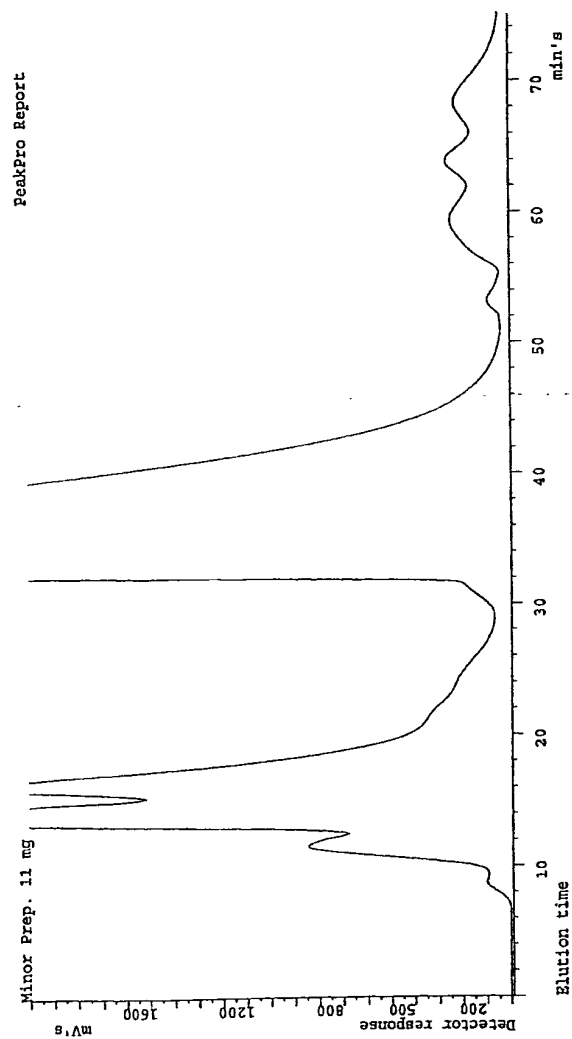


Fig. 5

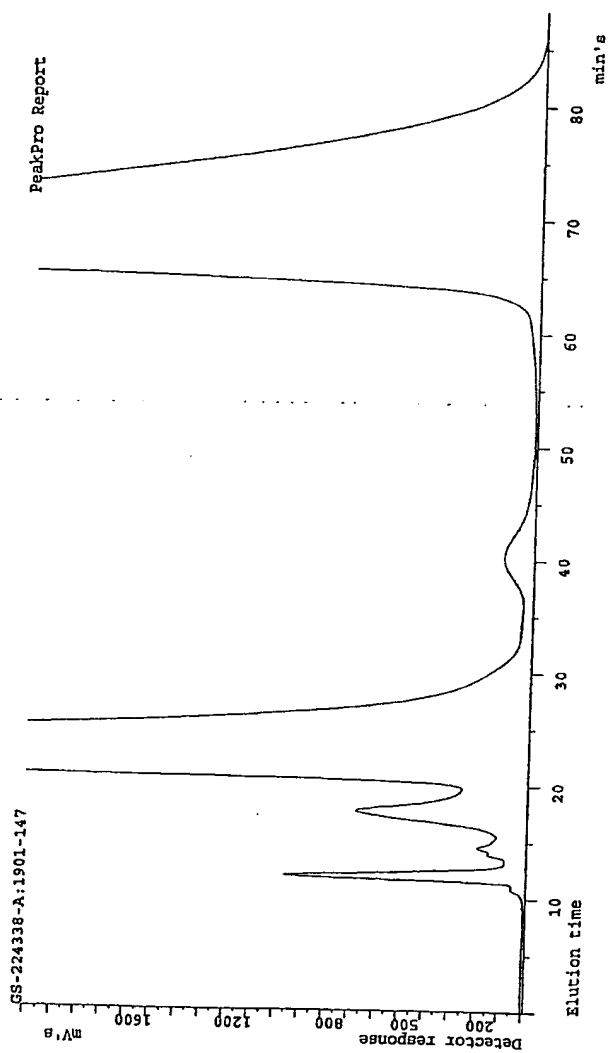


Fig. 6

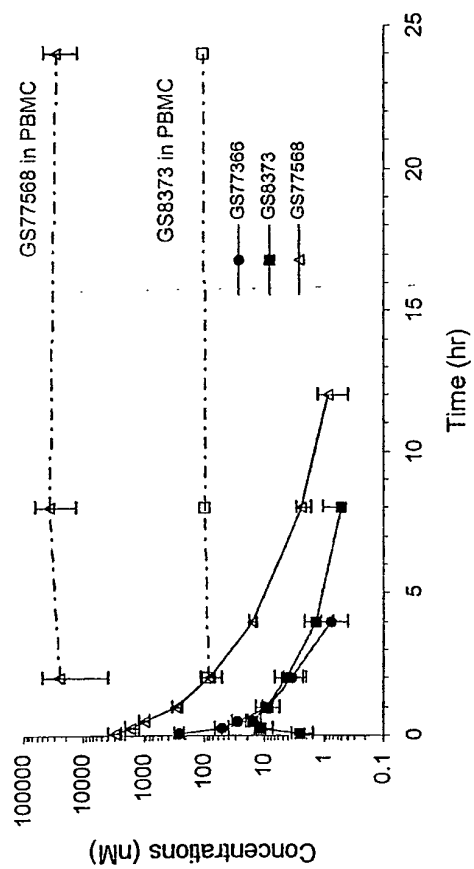


Fig. 7

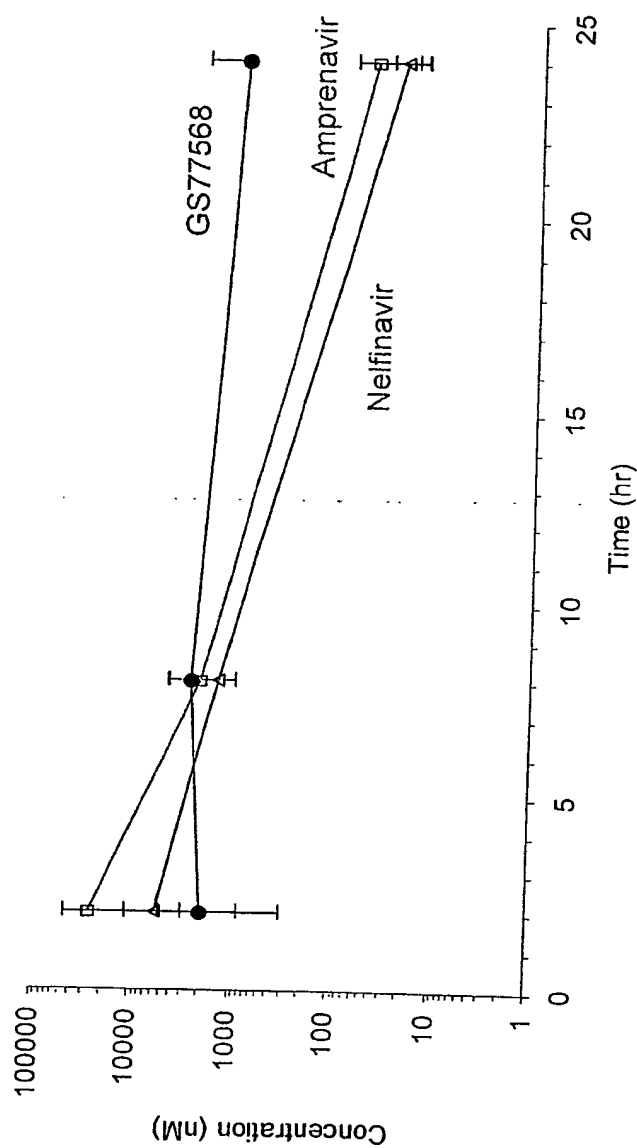


Fig. 8

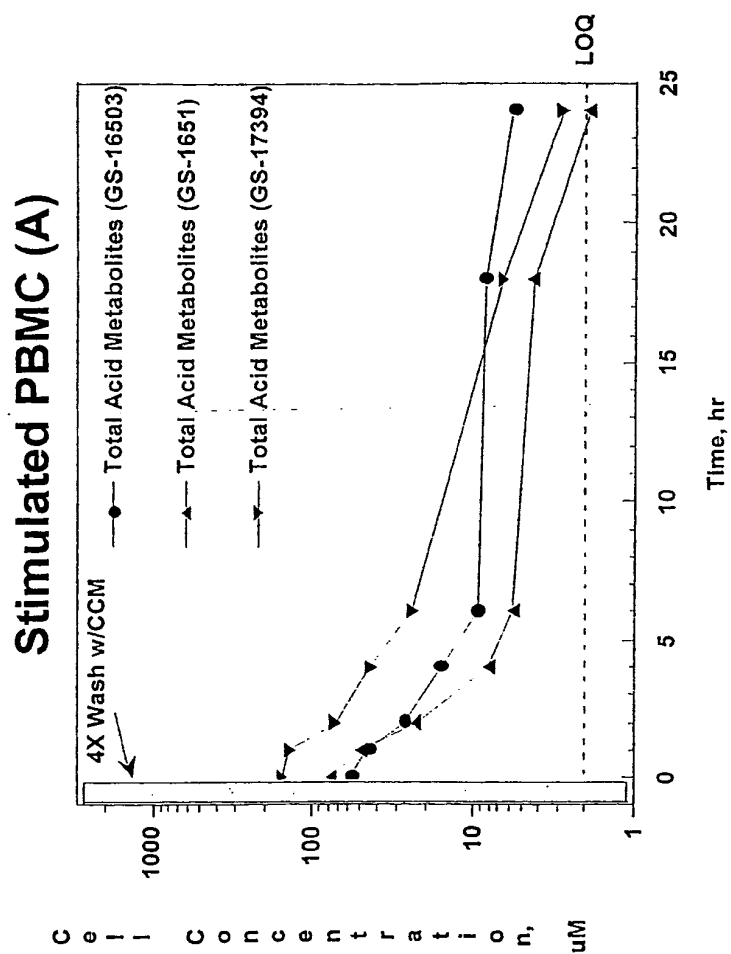


Fig. 9

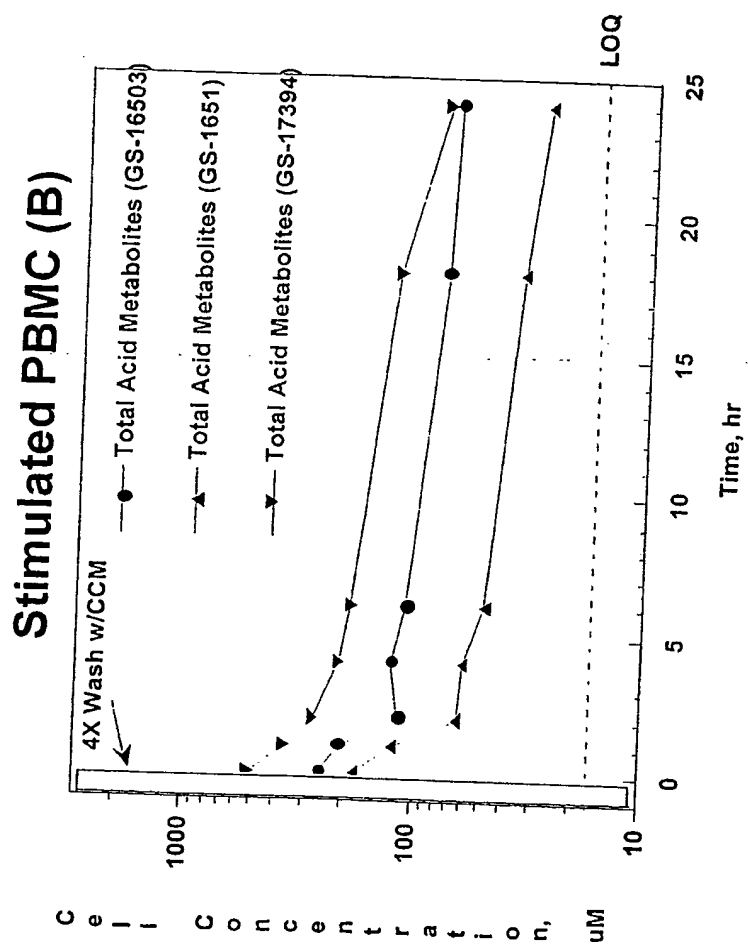


Fig. 10

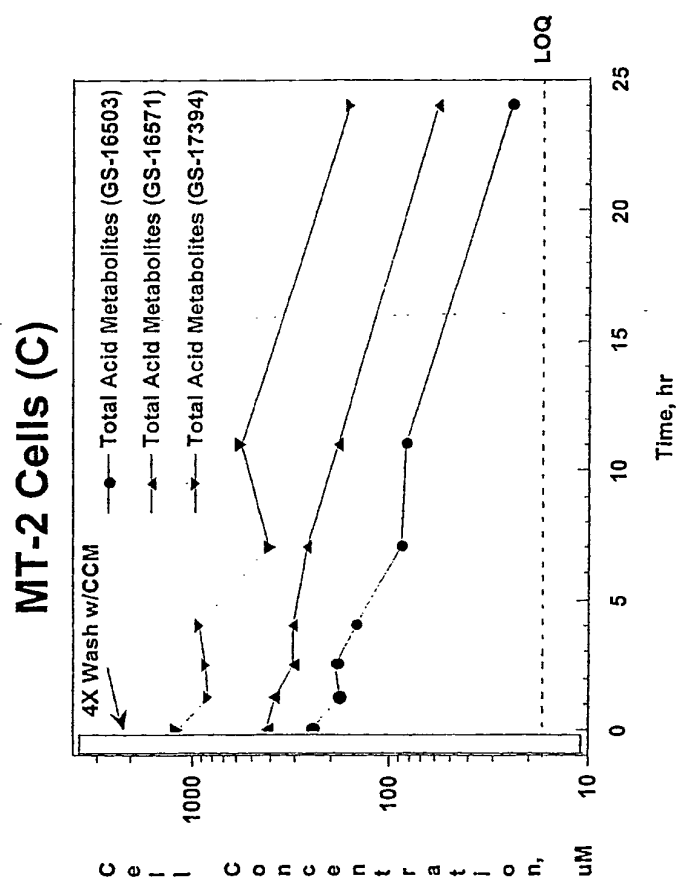
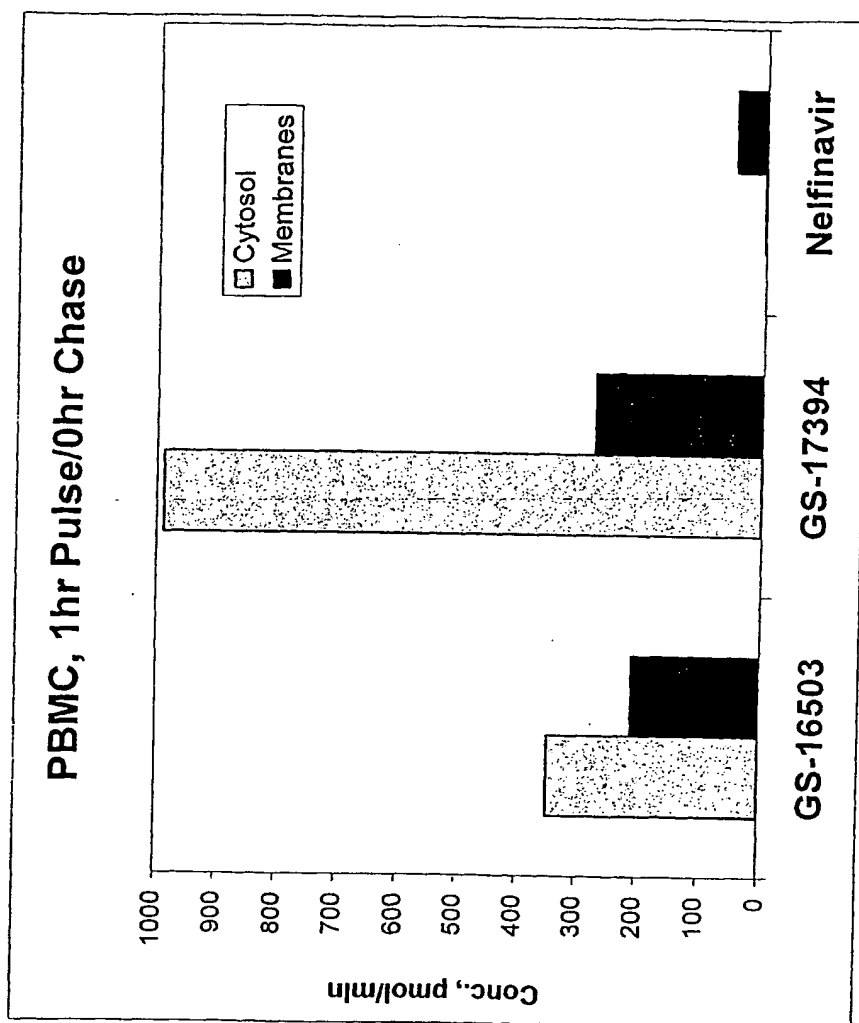
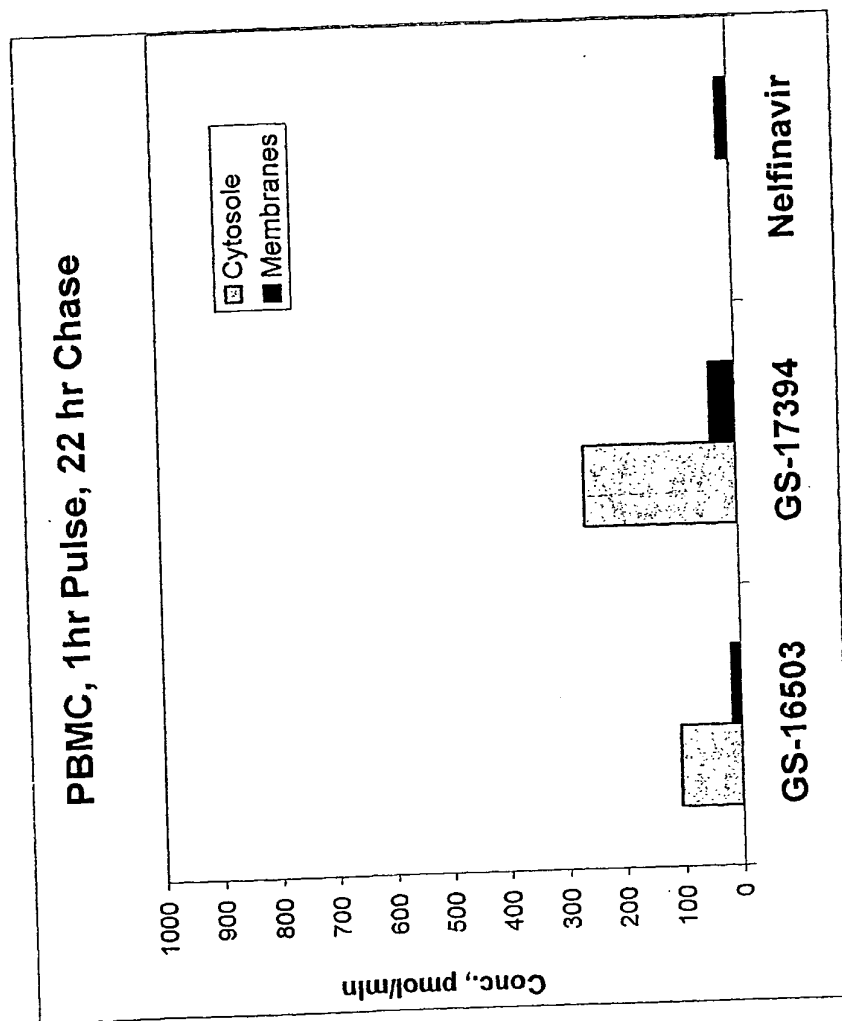




Fig. 11



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Fig. 12

SUBSTITUTE SHEET (RULE 26)

Fig. 13

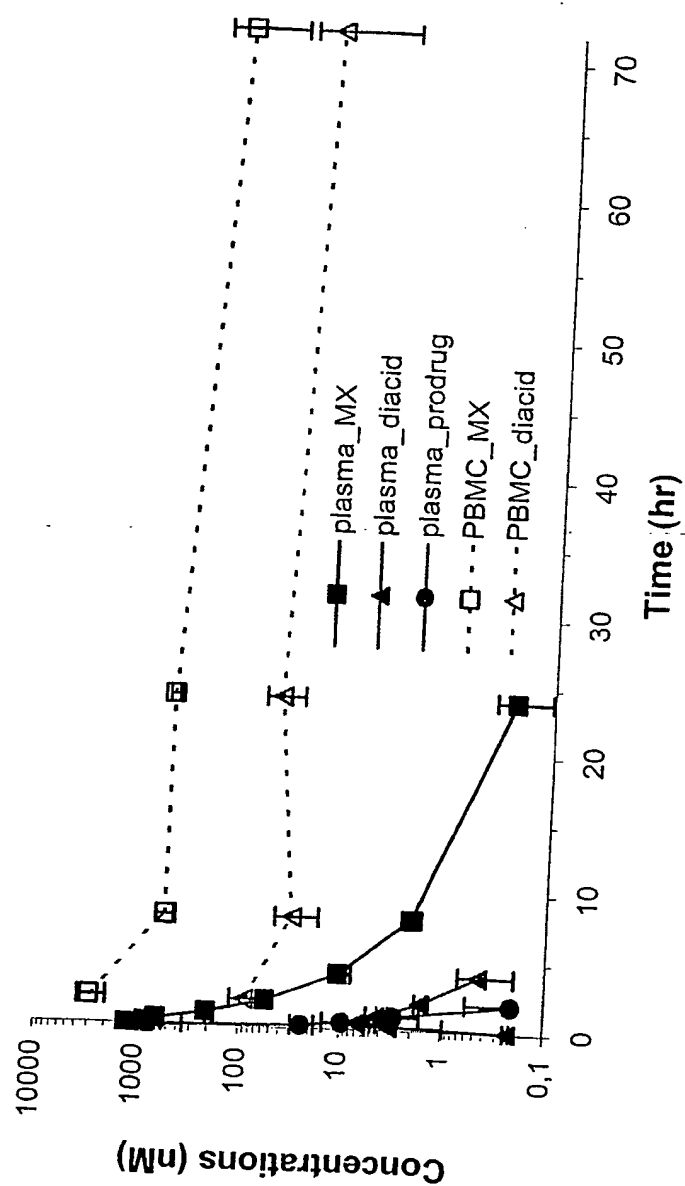


Fig. 14

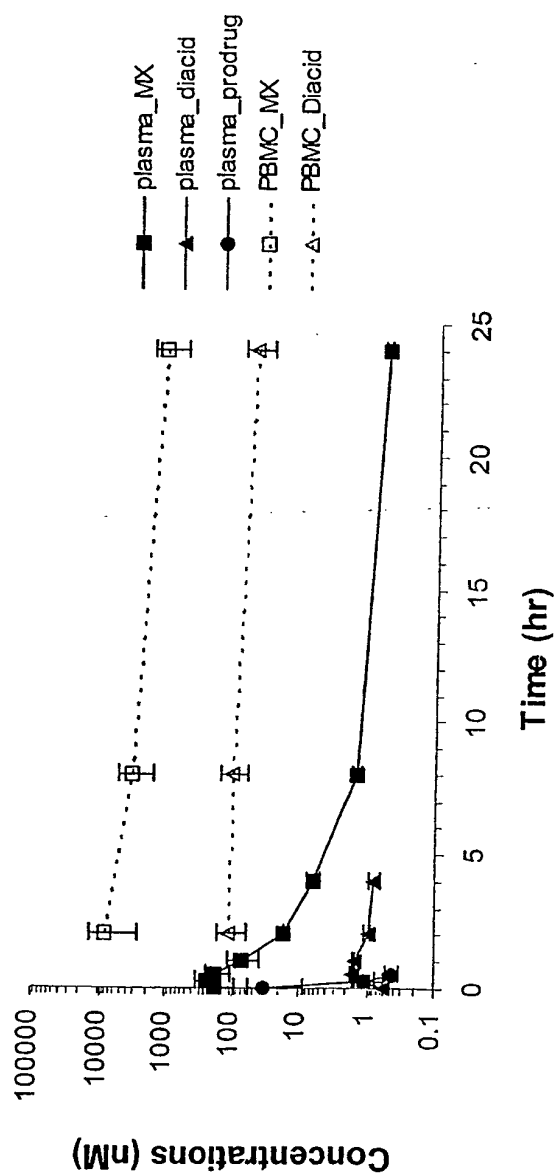


Fig. 15

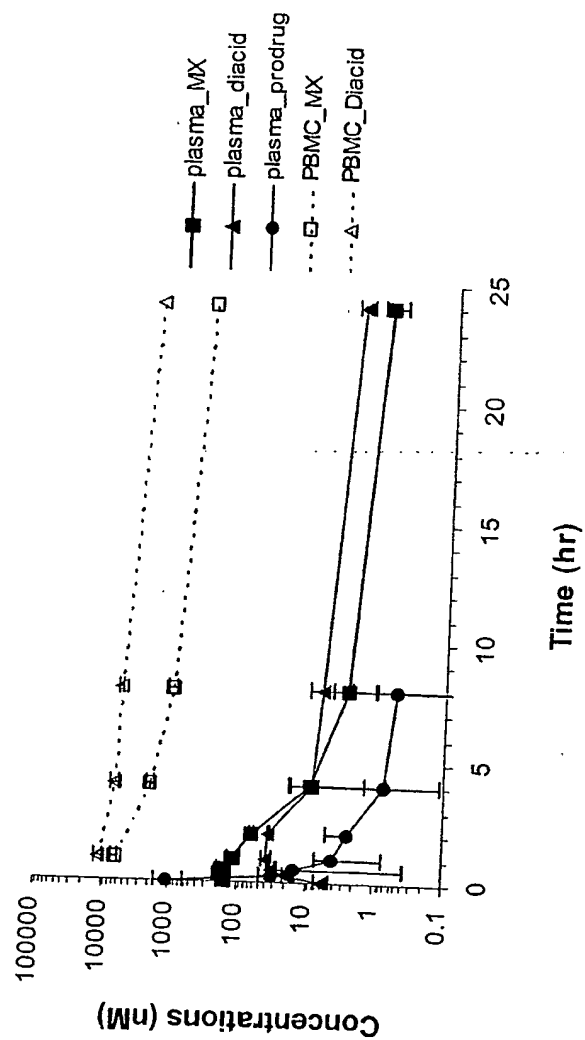


Fig. 16

